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(54) **VACCINE ANTIGENS FROM PISCIRICKETTSIA SALMONIS**

IMPFSTOFFANTIGENE AUS PISCIRICKETTSIA SALMONIS

ANTIGÈNES DE VACCIN PROVENANT DE LA PISCIRICKETTSIA SALMONIS

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WO-A-01/68865 **WO-A-2004/006953**
WO-A-2006/037383 **US-A1- 2003 165 526**

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Description

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a non-provisional application that claims priority under 35 U.S.C. § 119(e) of provisional applications U.S. Serial No. 61/014,782 filed December 19, 2007.

BACKGROUND OF THE INVENTION

Field of Invention:

[0002] The present invention relates to novel proteins from *Piscirickettsia salmonis.* The present invention also pertains to the nucleic acids that encode these proteins. The present invention further relates to a process of preparing a vaccine against salmonid rickettsial septicemia (SRS) using the proteins as antigens, or the nucleic acids in bacterial hosts to express such antigens. The present invention also relates to bacterins and viral antigens that can be combined to form a vaccine against SRS. The present invention also pertains to vaccines for preventing SRS, as well as preventing other bacterial and/or viral infections in fish.

Background:

[0003] Salmonid rickettsial septicemia (SRS), also known as piscirickettsiosis, is a fatal disease in salmonids. Although the etiological agent for SRS was identified in the late 1980's as *Piscirickettsia salmonis,* antibiotics proved to be an unsuccessful treatment, due, at least in part, to the intracellular nature of this bacterium [Bravo and Campos, FHS/AFS Newsl. 17:3 (1989); U.K. Patent Application 2 356 632]. As a consequence of the lack of a viable treatment, millions of farmed salmon die of SRS each year just in southern Chile alone [Smith et al., Dis. Aquat. Organ. 37(3):165-172 (1999)]. In addition, recent reports demonstrate a link between *Piscirickettsia*-like bacteria and disease syndromes in non-salmonid fish [see, Mauel and Miller, Veterin. Microbiol. 87(4):279-289 (2002)].

[0004] The Salmonidae family (salmonids) includes salmon, trout, char, and whitefish. Salmonids serve both as a food source and as a game fish. Moreover, in countries such as Chile, Norway, Canada, the United Kingdom, Ireland, and the United States, salmonids have become an important commercial product due, at least in part, to the ability of fish farmers to artificially spawn, incubate and raise the salmonids in captivity.

[0005] Unlike fish originating in the wild, those raised in captivity are amenable to prophylactic treatments such as vaccination. So far, several potential vaccines have been described, such as one based on a specific *Piscirickettsia salmonis* antigen, a 17 kDa lipoprotein OspA [U.K. Patent Application 2 356 632; *see also* WO 01/68865 A2]. Additional potential vaccines against *Piscirickettsia salmonis* are described by WO05035558 A2 and WO2006037383 A1. These published international patent applications describe an isolated *Piscirickettsia salmonis* ^{Ps}p45 protein and antigenic fragments thereof, as well as other SRS antigens.

[0006] The nucleotide coding sequence of the ^{Ps}p45 protein is included within SEQ ID NO: 16. The amino acid sequence of the ^{Ps}p45 protein within the deposited recombinant *Yersinia ruckeri* is SEQ ID NO: 7 [the amino acid sequence of SEQ ID NO: 8 is identical to that of SEQ ID NO: 7, except SEQ ID NO: 8 lacks the sequence for the signal peptide.]

[0007] Patent application WO 2004/006953 relates to vaccines for the protection of fish against P. salmonis, that comprise Anthrobacter cells and optionally a P. salmonis antigen. This publication is silent about $^{ps}P_1$90 and $^{ps}P_2$90.

[0008] A publication by Wilhelm, V. et al., in Vaccine 24: 5083-5091 (2006) identifies Hsp60, Hsp70 and FlgP as possible antigens suitable for use in vaccines for the protection of fish against P. salmonis. Wilhelm, V. et al., are silent about $^{ps}P_1$90 and $^{ps}P_2$90.

[0009] In addition to *Piscirickettsia salmonis,* other pathogens are known to cause disease in farmed fish, including salmon. One such pathogen is the Infectious Pancreatic Necrosis virus (IPN virus), which is an unenveloped, icosahedral, bisegmented dsRNA virus. The IPN virus contains one main structural protein, VP2 (52 kDa) and three additional proteins, VP1 (90 kDa), VP3 (30 kDa) and VP4 (28 kDa). VP2 is the main protein of the outer capsid and is therefore immunologically important in recognition and bonding of the virus. VP1 is thought to be a polymerase, whereas VP3 and VP4 are internal proteins. VP4 is believed to correspond to a form of VP3 fragment formed during viral differentiation [see, WO 02/38770 A1. Nucleotide and amino acid sequences for VP2 and VP3 have been determined [see, Havarstein et al., J. Gen.Virol. 71:299-308 (1990); Pryde et al., Archives of Vir. 129:287-293 (1992)].

[0010] There, therefore remains a need to provide new safe and effective vaccines against *Piscirickettsia salmonis.* In addition, there remains a need to identify new antigens from *Piscirickettsia salmonis* that can be used in such vaccines. Furthermore, there is a need to obtain nucleic acids that encode such antigens. In addition, there is a need to provide methods of vaccinating fish to protect them from *Piscirickettsia salmonis* and *Piscirickettsia*-like bacteria. Furthermore, there is a need to provide vaccines that can protect fish against *Piscirickettsia salmonis* and other unrelated pathogens,

particularly those of commercial importance, such as the IPN virus.

[0011] The citation of any reference herein should not be construed as an admission that such reference is available as "prior art" to the instant application.

SUMMARY OF THE INVENTION

[0012] The present invention provides an isolated, recombinant, or both isolated and recombinant protein from *Piscirickettsia salmonis,* as well as antigenic fragments thereof. The protein of the invention is $^{Ps}p_1 90$, a 90kDa protein (ORF1), as described below. Another 90kDa protein $^{Ps}p_2 90$, (ORF2) is described below. $^{Ps}p_1 90$ and $^{Ps}p_2 90$ can be expressed from any suitable nucleic acid that encodes one or both of them, respectively, *e.g.,* either DNA or RNA. In addition, $^{Ps}p_1 90$ or $^{Ps}p_2 90$ can be used as antigens in vaccines against SRS, either alone, or in combination with each other and/or other antigens.

[0013] The present invention further provides nucleic acids that encode the isolated and/or recombinant protein from *Piscirickettsia salmonis* and/or antigenic fragments of the protein. Furthermore, the present invention provides nucleotide probes and PCR primers that can be used, *e.g.,* to identify such nucleic acids that encode the protein of the invention. In addition, the present invention provides recombinant vectors that encode the *Piscirickettsia salmonis* protein of the present invention, or fragments thereof, such as recombinant viruses and bacteria. Corresponding attenuated or killed recombinant bacteria, *e.g.,* bacterins prepared from the recombant bacterial vectors are also provided.

[0014] The present invention further provides vaccines that comprise *Piscirickettsia salmonis* proteins of the present invention and/or antigenic fragments of these antigens. These antigens may be placed into a vaccine in any number of forms including as a recombinant protein itself, and/or as a recombinant protein expressed by a recombinant vector such as a recombinant gram negative bacterium, or as a naked DNA. In a particular embodiment of the present invention, the recombinant gram negative bacterium is a recombinant *E. coli* cell.

[0015] Preferably the recipient of a vaccine of the present invention receives protection from *Piscirickettsia salmonis.* In one embodiment, the vaccine comprises recombinant *E.coli* that encode and express the $^{Ps}p_1 90$ and optionally $^{Ps}p_2 90$ proteins and/or antigenic fragments of the $^{Ps}p_1 90$ and optionally $^{Ps}p_2 90$ protein. In a particular embodiment of this type, such recombinant *E.coli* are inactivated prior to being added to the vaccine and/or prior to the administration of the vaccine to the animal subject. In addition, booster vaccines are also provided by the present invention.

[0016] Antibodies that bind to the *Piscirickettsia salmonis* proteins of the present invention are also provided. Such antibodies can be used: to demonstrate the presence of, identify, and/or purify the proteins of the present invention.

[0017] Accordingly, the present invention provides a $^{Ps}p_1 90$ protein that comprises an amino acid sequence comprising at least 75% identity with the amino acid sequence of SEQ ID NO: 2. In another embodiment of this type the $^{Ps}p_1 90$ protein comprises an amino acid sequence comprising at least 90% identity with the amino acid sequence of SEQ ID NO: 2. In still another embodiment of this type the $^{Ps}p_1 90$ protein comprises an amino acid sequence comprising at least 95% identity with the amino acid sequence of SEQ ID NO: 2.

[0018] In yet another embodiment of this type the $^{Ps}p_1 90$ protein comprises an amino acid sequence comprising SEQ ID NO: 2 that comprises one or more conservative amino acid substitutions. In still another embodiment the $^{Ps}p_1 90$ protein comprises an amino acid sequence comprising SEQ ID NO: 2 that comprises one to ten amino acid substitutions. In a particular embodiment of this type the $^{Ps}p_1 90$ protein comprises an amino acid sequence of SEQ ID NO: 2 that comprises one to ten conservative amino acid substitutions. In a specific embodiment, the $^{Ps}p_1 90$ protein comprises the amino acid sequence of SEQ ID NO: 2. In another embodiment, the $^{Ps}p_1 90$ protein consists essentially of the amino acid sequence of SEQ ID NO: 2. *P. salmonis* variants of the $^{Ps}p_1 90$ protein that comprises the amino acid sequence of SEQ ID NO: 2 are also included as part of the present invention.

[0019] Preferably, all of the $^{Ps}p_1 90$ proteins of the present invention bind to an antibody elicited by the $^{Ps}p_1 90$ protein that has the amino acid sequence of SEQ ID NO: 2. More preferably, when a $^{Ps}p_1 90$ protein of the present invention is included as an antigen in a vaccine administered to salmonids, the vaccine provides protection against SRS to the vaccinated salmonids.

[0020] Also described is a $^{Ps}p_2 90$ protein that comprises an amino acid sequence comprising at least 60% identity with the amino acid sequence of SEQ ID NO: 4. In particular the $^{Ps}p_2 90$ protein comprises an amino acid sequence comprising at least 75% identity with the amino acid sequence of SEQ ID NO: 4. More particularly the $^{Ps}p_2 90$ protein comprises an amino acid sequence comprising at least 90% identity with the amino acid sequence of SEQ ID NO: 4. More particularly the $^{Ps}p_2 90$ protein comprises an amino acid sequence comprising at least 95% identity with the amino acid sequence of SEQ ID NO: 4.

[0021] It is described herein that the $^{Ps}p_2 90$ protein comprises an amino acid sequence comprising SEQ ID NO: 4 that comprises one or more conservative amino acid substitutions. In particular the $^{Ps}p_2 90$ protein comprises an amino acid sequence comprising SEQ ID NO: 4 that comprises one to ten amino acid substitutions. More particularly the $^{Ps}p_2 90$ protein comprises an amino acid sequence of SEQ ID NO: 4 that comprises one to ten conservative amino acid substitutions. More particularly the $^{Ps}p_2 90$ protein comprises the amino acid sequence of SEQ ID NO: 4. More particularly the

$^{Ps}p_2$90 protein consists essentially of the amino acid sequence of SEQ ID NO: 4. *P. salmonis* variants of the $^{Ps}p_2$90 protein that comprises the amino acid sequence of SEQ ID NO: 4 are also included as part of the present invention.

[0022] Preferablv. all of the $^{Ps}p_2$90 proteins bind to an antibody elicited by the $^{Ps}p_2$90 protein that has the amino acid sequence of SEQ ID NO: 4. More preferably, when a $^{Ps}p_2$90 protein is included as an antigen in a vaccine administered to salmonids, the vaccine provides protection against SRS to the vaccinated salmonids.

[0023] The present invention also provides antigenic fragments of all of the *P. salmonis* protein of the present invention. In a particular embodiment, the antigenic fragment is of the $^{Ps}p_1$90 protein having the amino acid sequence of SEQ ID NO: 2. Also described is that the antigenic fragment is of the $^{Ps}p_2$90 protein having the amino acid sequence of SEQ ID NO: 4. The antigenic fragments of the present invention can be in any form including but not limited to: isolated, recombinant, chemically synthesized, both recombinant and isolated, or both chemically synthesized and isolated.

[0024] The present invention further provides chimeric polypeptides that comprise a $^{Ps}p_1$90 protein of the present invention or antigenic fragment thereof, and optionally a $^{Ps}p_2$90 protein or antigenic fragment thereof. In one such embodiment, the chimeric polypeptide comprises the $^{Ps}p_1$90 protein having the amino acid sequence of SEQ ID NO: 2. Also described is that the chimeric polypeptide comprises the $^{Ps}p_2$90 protein having the amino acid sequence of SEQ ID NO: 4. In another embodiment, the chimeric polypeptide comprises an antigenic fragment of a $^{Ps}p_1$90 protein of the present invention that has the amino acid sequence of SEQ ID NO: 2. Also described is that the chimeric polypeptide comprises an antigenic fragment of the $^{Ps}p_2$90 protein that comprises the amino acid sequence of SEQ ID NO: 4. The chimeric polypeptides of the present invention can be in any form including but not limited to: isolated, recombinant, chemically synthesized, both recombinant and isolated, or both chemically synthesized and isolated.

[0025] The present invention further provides antibodies elicited by the $^{Ps}p_1$90 protein of the present invention, including those elicited by a chimeric polypeptide of the present invention. In one embodiment, the antibody is solicited by the $^{Ps}p_1$90 protein having the amino acid sequence of SEQ ID NO: 2.

[0026] The present invention also provides antibodies solicited by an antigenic fragment of a $^{Ps}p_1$90 protein of the present invention. In one such embodiment, the antibody is solicited by an antigenic fragment of a $^{Ps}p_1$90 protein of the present invention having the amino acid sequence of SEQ ID NO: 2.

[0027] In another aspect of the present invention, nucleic acids are provided which encode: the $^{Ps}p_1$90 proteins, the $^{Ps}p_2$90 proteins, the antigenic fragments of these $^{Ps}p_1$90 proteins and/or $^{Ps}p_2$90 proteins, and/or the corresponding chimeric polypeptides of the present invention. Any of these nucleic acids can further comprise heterologous nucleotide sequences. The nucleic acids of the present invention can be in any form including but not limited to: isolated, recombinant, chemically synthesized, both recombinant and isolated, or both chemically synthesized and isolated.

[0028] In a particular embodiment, a nucleic acid of the present invention encodes a $^{Ps}p_1$90 protein that comprises the amino acid sequence of SEQ ID NO: 2. In a particular embodiment of this type the nucleic acid comprises the nucleotide sequence of SEQ ID NO: 1. In an alternative embodiment the nucleic acid comprises the nucleotide sequence of SEQ ID NO: 5.

[0029] Also described is a nucleic that encodes a $^{Ps}p_2$90 protein that comprises the amino acid sequence of SEQ ID NO: 4. In particular the nucleic acid comprises the nucleotide sequence of SEQ ID NO: 3. More particularly the nucleic acid comprises the nucleotide sequence of SEQ ID NO: 6.

[0030] The present invention also provides nucleic acids (e.g., DNA molecules) of 18 nucleotides or more that hybridize under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 1. In a particular embodiment, the nucleic acid comprises 120 nucleotides or more and hybridizes under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 1. In another embodiment, the nucleic acid comprises 300 nucleotides or more and hybridizes under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 1. In still another embodiment, the nucleic acid comprises 900 nucleotides or more and hybridizes under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 1. In yet another embodiment the nucleic acid comprises between 2000 to 3000 nucleotides and hybridizes under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 1. In a related embodiment, the DNA molecule encodes a $^{Ps}p_1$90 protein and hybridizes under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 1.

[0031] Also described are nucleic acids (*e.g.*, DNA molecules) of 18 nucleotides or more that hybridize under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 3. In particular the nucleic acid comprises 120 nucleotides or more and hybridizes under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 3. More particularly the nucleic acid comprises 300 nucleotides or more and hybridizes under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 3. More particularly the nucleic acid comprises 900 nucleotides or more and hybridizes under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 3. More particularly the nucleic acid comprises between 1500 to 2600 nucleotides and hybridizes under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 3. Also described is that the DNA molecule encodes a $^{Ps}p_2$90 protein and hybridizes under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 3.

[0032] The present invention also provides vectors that comprise one or more of the nucleic acids of the present

invention. In one embodiment of this type, the vector is an expression vector. Preferably the nucleic acids of the present invention are operatively linked to a transcriptional control sequence in the expression vectors.

[0033] The expression vectors of the present invention can be used to express one or more $^{Ps}p_1 90$ proteins, antigenic fragments of the $^{Ps}p_1 90$ proteins and optionally $^{Ps}p_2 90$ proteins, and/or corresponding chimeric polypeptides. In one such embodiment, the expression vector is a plasmid that can function in *E. coli.* In a particular embodiment, the expression vector is the EGT1 plasmid. In one such embodiment, the expression vector is an EGT1 plasmid that expresses $^{Ps}p_1 90$ that has the amino acid sequence of SEQ ID NO: 2, and which has the BCCM accession No. LMBP 5690. Also described is that the expression vector is an EGT1 plasmid that expresses $^{Ps}p_2 90$ that has the amino acid sequence of SEQ ID NO: 4, and which has the BCCM accession No. LMBP 5691.

[0034] The present invention further provides host cells that comprise the vectors of the present invention. In a particular embodiment, the host cell expresses one or more $^{Ps}p_1 90$ proteins, antigenic fragments of the $^{Ps}p_1 90$ proteins and optionally $^{Ps}p_2 90$ proteins, and/or corresponding chimeric polypeptides. In one embodiment the host cell comprises a plasmid that expresses $^{Ps}p_1 90$ that comprises the amino acid sequence of SEQ ID NO: 2. In a particular embodiment of this type, the plasmid is an EGT1 plasmid that has the BCCM accession No. LMBP 5690. Also described is that the host cell comprises a plasmid that expresses $^{Ps}p_2 90$ that comprises the amino acid sequence of SEQ ID NO: 4. In a particular embodiment of this type, the plasmid is an EGT1 plasmid that has the BCCM accession No. LMBP 5691. Preferably, the host cell is an *E. coli* cell.

[0035] The present invention also provides methods for expressing and/or producing a one or more $^{Ps}p_1 90$ proteins, antigenic fragments of the $^{Ps}p_1 90$ proteins and optionally $^{Ps}p_2 90$ proteins, and/or corresponding chimeric polypeptides. One such embodiment is culturing a host cell of the present invention in a culture medium. In a particular embodiment, the method further comprises isolating the $^{Ps}p_1 90$ protein(s), antigenic fragment(s) of the $^{Ps}p_1 90$ protein(s) and optionally $^{Ps}p_2 90$ protein(s), and/or corresponding chimeric polypeptide(s). In one such embodiment, the host cell is an *E. coli* cell. In a particular embodiment the host cell comprises a EGT1 plasmid that expresses $^{Ps}p_1 90$ that comprises the amino acid sequence of SEQ ID NO: 2. In a particular embodiment of this type, the plasmid is an EGT1 plasmid that has the BCCM accession No. LMBP 5690. Also described is that the host cell comprises a EGT1 plasmid that expresses $^{Ps}p_2 90$ that comprises the amino acid sequence of SEQ ID NO: 4. In particular the plasmid is an EGT1 plasmid that has the BCCM accession No. LMBP 5691.

[0036] In another aspect of the present invention, immunogenic compositions are provided comprising the proteins, and/or antigenic fragments, and/or recombinant host cells, and/or bacterins of the present invention. In a preferred embodiment of this type, an immunogenic composition of the present invention is a vaccine. Accordingly, the vaccines of the present invention can comprise any of the immunogenic compositions of the present invention. Preferred vaccines protect fish against SRS, either alone or in multivalent vaccines that may also protect against other pathogens. In a related embodiment, a vaccine is a naked DNA vaccine that comprises a recombinant DNA vector that comprises an antigen of the present invention or an antigenic fragment thereof.

[0037] Immunogenic compositions of the present invention comprise antigenically effective amounts of a $^{Ps}p_1 90$ protein of the present invention and/or of an antigenic fragment thereof; and/or antigenically effective amounts of a mixture of a $^{Ps}p_1 90$ protein of the present invention and/or of an antigenic fragment thereof and a $^{Ps}p_2 90$ protein of the present invention and/or of an antigenic fragment thereof.

[0038] In a particular embodiment, the immunogenic composition comprises a bacterin that comprises the plasmid having the BCCM accession No. LMBP 5690. In another embodiment, the the immunogenic composition comprises both a bacterin that comprises the plasmid having the BCCM accession No. LMBP 5690 and a bacterin that comprises the plasmid having the BCCM accession No. LMBP 5691.

[0039] The vaccines of the present invention can further include an adjuvant. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, and dinitrophenol.

[0040] A vaccine and/or immunogenic composition of the present invention can further comprise one or more additional *P. salmonis* proteins or an antigenic fragment thereof. In one such embodiment the *P. salmonis* protein is the $^{Ps}45$ protein. In a particular embodiment of this type, the $^{Ps}45$ protein can comprise the amino acid sequence of SEQ ID NO: 8.

[0041] In another embodiment, a vaccine of the present invention further comprises one or more antigens obtained from an Infectious Pancreatic Necrosis (IPN) virus. These recombinant proteins are preferably expressed by transformed yeast, *Pichia pastoris.* In one such embodiment, the antigen obtained from the IPN virus is the VP2 var protein or antigenic fragment thereof. In another embodiment the antigen obtained from the IPN virus is the VP3 protein or antigenic fragment thereof. In a preferred embodiment, the vaccine comprises both the VP2 var protein or antigenic fragment thereof and the VP3 protein or antigenic fragment thereof.

[0042] In still another embodiment a vaccine of the present invention comprises one or more antigens obtained from *Aeromonas salmonicida.* In a particular embodiment, the *Aeromonas salmonicida* comprising the antigens is prepared from a culture grown under iron-depleted conditions. In another embodiment, the *Aeromonas salmonicida* comprising

the antigens is prepared from a culture grown under iron-supplemented conditions. In a preferred embodiment, two sets of *Aeromonas salmonicida* antigens are employed in the vaccine, one set from a culture grown under iron-depleted conditions the other set from a culture grown under iron-supplemented conditions. In a particular embodiment, a multivalent vaccine comprises antigens from *Piscirickettsia salmonis,* IPN, and *Aeromonas salmonicida.*

[0043] The present invention also provides methods of protecting a fish from salmonid rickettsial septicemia (SRS), or SRS along with one or more other pathogenic disease(s) through the vaccination of the fish with a vaccine of the present invention. In a particular embodiment the other disease is Infectious Pancreatic Necrosis. In another embodiment the other disease is furunculosis. In still another embodiment the method of protecting the fish includes protecting against SRS, Infectious Pancreatic Necrosis, and furunculosis (caused by *Aeromonas salmonicida*).

[0044] The vaccines of the present invention can be administered by any method. In one embodiment a vaccine of the present invention is administered by immersion. In another embodiment a vaccine of the present invention is administered by injection. In yet another embodiment a vaccine of the present invention is administered by oral administration.

[0045] In addition, related booster vaccines are also provided by the present invention. The administration of a given booster vaccine is preferably performed through oral administration.

[0046] Any fish may be the recipient of the vaccines of the present invention. Examples of recipient fish are listed below. In a particular embodiment, the fish is a teleost. In a preferred embodiment, the telost is a salmonid. In a more preferred embodiment the salmonid is a salmon. In one such embodiment the salmon is a *Salmo salar* (Atlantic salmon). In another embodiment the salmon is an *Oncorhynchus kisutch* (coho salmon). In yet another embodiment the salmonid is an *Oncorhynchus mykiss* (rainbow trout).

[0047] Accordingly, it is a principal object of the present invention to provide a vaccine that protects salmonids against SRS.

[0048] It is a further object of the present invention to provide a vaccine that protects fish from salmonid rickettsial septicemia (SRS) and Infectious Pancreatic Necrosis (IPN).

[0049] It is a further object of the present invention to provide an effective way to protect against assorted fish infections by providing a multivalent vaccine.

[0050] It is a further object of the present invention to provide a protocol that can lead to the successful vaccination of fish in captivity.

[0051] It is a further object of the present invention to provide a DNA construct that encodes the $^{Ps}p_1 90$ protein or variant thereof.

[0052] It is a further object of the present invention to provide a polypeptide having an amino acid sequence of SEQ ID NO: 2, or an antigenic fragment thereof.

[0053] It is a further object of the present invention to provide a recombinant subunit vaccine against SRS.

[0054] It is a further object of the present invention to provide inactivated recombinant bacterial vectors encoding specific antigens to be used in vaccines against SRS.

[0055] These and other aspects of the present invention will be better appreciated by reference to the following drawings and Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0056] Figure 1 illustrates the cumulative percent mortality in different test groups of fish after vaccination. The curves show the results of the saline controls denoted by diamonds, the adjuvant controls denoted by squares, the 90 kDa ORF 1 [$^{Ps}p_1 90$] vaccine denoted by triangles, and the 90 kDa ORF 2 [$^{Ps}p_2 90$] vaccine denoted by "X"'s.

DETAILED DESCRIPTION OF THE INVENTION

[0057] The present invention provides safe and effective vaccines to protect fish against *Piscirickettsia salmonis* infections. In addition, the present invention provides methods of vaccinating fish to protect them from *Piscirickettsia salmonis* and *Piscirickettsia*-like bacteria. Moreover, the present invention provides vaccines that can protect vaccinated fish from *Piscirickettsia salmonis* and other unrelated pathogens, such as the IPN virus. Methods of making the vaccines of the present invention are also provided. The vaccines of the present invention (including booster vaccines) can be administered to fish by a number of means including by immersion, by injection, and/or through oral administration.

[0058] Notably, US Published Patent Application No. US20070207165 (A1) and WO2006037383(A1) specifically teach the use a recombinant *Yersinia ruckeri* vector to express *P. salmonis* proteins, and indeed, exemplify the successful use of *Yersinia ruckeri* to express the $^{Ps}p45$ protein. However, after considerable time and effort, *Yersinia ruckeri* proved to be an inappropriate host cell for expressing either $^{Ps}p_1 90$ or $^{Ps}p_2 90$ due both to instability, and the inability to demonstrate expression of these proteins. Surprisingly, and contrary to the earlier teachings of US20070207165 (A1) and WO2006037383(A1), *E.coli* cells proved to be the preferred recombinant host cell to express either $^{Ps}p_1 90$ or $^{Ps}p_2 90$.

[0059] Accordingly, in a particular aspect of the present invention the $^{Ps}p_1$90 and $^{Ps}p_2$90 proteins are expressed in recombinant *E. coli* host cells containing pEGT1 plasmids. Two corresponding recombinant *E. coli* HMS174(DE3)/pEGT1 plasmids encoding these proteins were deposited with the:

Belgian Coordinated Collections of Microorganisms (BCCM) under the terms of the Budapest Treaty and represented by:

BCCM/LMBP
Department of Molecular Biology
Ghent University
Fiers-Schell-Van Mantagu Building
Technologiepark 927
B-9052 Zwijnaarde

The International Depository Authority :

Belgian Coordinated Collections of Microorganisms (BCCM™) Laboratorium voor Moleculaire Biologie-Plasmidencollectie (LMBP) Universiteit Gent
Technologiepark 927
B-9052 Gent-Zwijnaarde, Belgium

Both plasmid deposits were all made on October 19, 2007.

[0060]

- **$^{Ps}p_1$90**
  - *E. coli* HMS174(DE3)/pEGT1/AL-ORF1-90kDa
  - BCCM accession No. LMBP 5690
- **$^{Ps}p_2$90**
  - *E. coli* HMS174(DE3)/pEGT1/AL-ORF2-90kDa
  - BCCM accession No. LMBP 5691

[0061] The present invention also provides vaccines against SRS that further comprise and/or encode one or more additional *P. salmonis* antigens. Such additional antigens include those described by US Published Patent Application No. US20070207165 (A1) and those described by WO2006037383(A1). These antigens include isolated *P. salmonis* ^{Ps}p45 protein comprising the amino acid sequence of SEQ ID NO: 7 (complete ^{Ps}p45 protein) or SEQ ID NO: 8 (^{Ps}p45 protein without the signal sequence) and antigenic fragments thereof.

[0062] Additional antigens described by US20070207165 (A1) and WO2006037383(A1), *see also* Table 14 below, include those comprising the amino acid sequence of SEQ ID NO: 9 (which shows homology with a protein family coding for AMP-binding enzymes), SEQ ID NO: 10 (which shows no homology to any protein family), SEQ ID NO: 11 (which shows no homology to any protein family), SEQ ID NO: 12 (which shows homology to the DDE endonuclease family and in particular to the integrase core domain), SEQ ID NO: 13 (which shows homology to transposases), SEQ ID NO: 14 (which shows some homology to the HlyD family of secretory proteins), and/or SEQ ID NO: 15 (which shows homology to the intergral membrane AcrB/AcrD/ AcrB protein family). These antigens can be expressed *e.g.*, by nucleic acids that encode one or more of these amino acid sequences.

[0063] As indicated above, ^{Ps}p45 protein can be produced by recombinant *Yersinia ruckeri* cells.

[0064] The present invention also provides combination vaccines against SRS and IPN (SRS/IPN vaccines) that comprise one or more inventive *P. salmonis* 90kDa antigens (*e.g.*, $^{Ps}p_1$90 and optionally $^{Ps}p_2$90) optionally in combination with any of the ^{Ps}p45 proteins or SRS antigens noted above, in combination with one or more antigens obtained from an Infectious Pancreatic Necrosis (IPN) virus. These recombinant proteins (IPN antigens) are preferably expressed by transformed yeast, *Pichia pastoris.*

[0065] In one such embodiment, the antigen obtained from the IPN virus is the VP2 var protein or an antigenic fragment thereof.

[0066] As used herein the following terms shall have the definitions set out below:

As used herein the term "$^{Ps}p_1$90" is used interchangably with the term "ORF1" and denotes a specific *Piscirickettsia salmonis* protein that is about 90kDa in molecular weight. In a particular embodiment, $^{Ps}p_1$ 90 comprises the amino acid sequence of SEQ ID NO: 2, which is encoded by the *P. salmonis* nucleotide sequence SEQ ID NO: 1 and the nucleotide sequence SEQ ID NO: 5, which was optimized for *E. coli codon* usage.

$^{Ps}p_1$ 90 is encoded by an EGT1 plasmid deposited with the BCCM™/LMBP Collection having ascension number LMP 5690.

As used herein the term "$^{Ps}p_2$90" is used interchangably with the term "ORF2" and denotes a specific *Piscirickettsia salmonis* protein that is about 90kDa in molecular weight. Described herein $^{Ps}p_2$90 comprises the amino acid sequence of SEQ ID NO: 4, which is encoded by the *P. salmonis* nucleotide sequence SEQ ID NO: 3 and the nucleotide sequence SEQ ID NO: 6, which was optimized for *E. coli* codon usage.

$^{Ps}p_2$ 90 is encoded by an EGT1 plasmid deposited with the BCCM™/LMBP Collection having ascension number LMP 5691.

[0067] As used herein the term "polypeptide" is used interchangeably with the term "protein" and is further meant to encompass peptides. Therefore, as used herein, a polypeptide is a polymer of two or more amino acids joined together by peptide linkages. Preferably, a polypeptide is a polymer comprising twenty or more amino acid residues joined together by peptide linkages, whereas a peptide comprises two to twenty amino acid residues joined together by peptide linkages.

[0068] As used herein a polypeptide "consisting essentially of" or that "consists essentially of" a specified amino acid sequence is a polypeptide that (i) retains an important characteristic of the polypeptide comprising that amino acid sequence, e.g., the antigenicity of at least one epitope of the inventive 90kDa protein(s), and (ii) further comprises the identical amino acid sequence(s), except it consists of plus or minus 10% (or a lower percentage), and preferably plus or minus 5% (or a lower percentage) of the amino acid residues. In a particular embodiment, additional amino acid residues included as part of the polypeptide are part of a linked Tag, such as a C-terminal His_6 Tag.

[0069] A molecule is "antigenic" when it is capable of specifically interacting with an antigen recognition molecule of the immune system, such as an immunoglobulin (antibody) or T cell antigen receptor. An antigenic polypeptide (and/or fragment of the polypeptide) contains at least 6, and preferably at least 12 or more amino acid residues. An antigenic portion of a molecule can be that portion that is immunodominant for recognition by an antibody or a T cell receptor, and/or it can be a portion used to generate an antibody to the molecule by conjugating an immunogenic portion of the antigen to a carrier molecule for immunization. A molecule that is antigenic need not be itself immunogenic, *i.e.,* capable of eliciting an immune response without a carrier.

[0070] As used herein the term "antigenic fragment" of a particular protein is a fragment of that protein that is antigenic. For example, an antigenic fragment of a $^{Ps}p_1$90 protein or a $^{Ps}p_2$90 protein can be any antigenic fragment of the $^{Ps}p_1$90 protein or $^{Ps}p_2$90 protein respectively, including large fragments that are missing as little as a single amino acid from the full-length protein. In a particular embodiment, an antigenic fragment of the $^{Ps}p_1$90 protein or a $^{Ps}p_2$90 protein contains between 12 and 800 amino acid residues. In another embodiment, an antigenic fragment of the $^{Ps}p_1$90 protein or a $^{Ps}p_2$90 protein contains between 25 and 250 amino acid residues. In yet another embodiment, an antigenic fragment of a $^{Ps}p_1$90 protein or a $^{Ps}p_2$90 protein contains 100 amino acid residues or more, but fewer than 600 amino acid residues. In still another embodiment, an antigenic fragment of a $^{Ps}p_1$90 protein or a $^{Ps}p_2$90 protein contains 250 amino acid residues or more, but fewer than 600 amino acid residues. In yet another embodiment, an antigenic fragment of a $^{Ps}p_1$90 protein or a $^{Ps}p_2$90 protein contains 400 amino acid residues or more, but fewer than 600 amino acid residues.

[0071] An antigenic fragment of a given $^{Ps}p_1$90 protein or a $^{Ps}p_2$90 protein can be obtained from a recombinant source, from a protein isolated from natural sources, or through chemical synthesis. Similarly, an antigenic fragment can be obtained following the proteolytic digestion of such $^{Ps}p_1$90 proteins, $^{Ps}p_2$90 proteins or fragments of either. Alternatively, an antigenic fragment of the present invention can be generated by recombinant expression, or alternatively, through peptide synthesis.

[0072] As used herein, a multivalent vaccine is a vaccine that comprises two or more different antigens. In a particular embodiment of this type, the multivalent vaccine stimulates the immune system of the recipient against two or more different pathogens. Specific multivalent vaccines are exemplified below.

[0073] As used herein the term "chimeric protein" is used interchangeably with the terms "chimeric polypeptide" and "chimeric peptide" and is meant to include fusion proteins, polypeptides, and peptides. A "chimeric protein" comprising a $^{Ps}p_1$90 and/or $^{Ps}p_2$90 protein of the present invention comprises at least a portion of a particular protein (*e.g.,* $^{Ps}p_1$90) joined *via* a peptide bond to at least a portion of a different protein (*e.g.*, a non-$^{Ps}p_1$90 protein). A chimeric protein of the present invention also can comprise two or more different proteins and/or portions thereof, including a chimeric $^{Ps}p_1$90-$^{Ps}p_2$90 protein. Chimeric proteins of the present invention also can have additional structural, regulatory, and/or catalytic properties. As used herein a chimeric protein can contain multiple additions to at least a portion of a given protein, *e.g.*, a chimeric protein can comprise both a His_6Tag and an alternative signal sequence. In a particular embodiment, a non-$^{Ps}p_1$90 (or non- $^{Ps}p_2$90) portion of the chimeric protein functions as a means of detecting and/or isolating the chimeric protein or fragment thereof after a recombinant nucleotide encoding the given protein or antigenic fragment thereof is

expressed. Non- $^{Ps}p_1$90 (or non- $^{Ps}p_2$90) protein amino acid sequences are generally, but not always, either amino- or carboxy-terminal to the protein sequence.

[0074] As used herein one amino acid sequence is 100% "identical" to a second amino acid sequence when the amino acid residues of both sequences are identical. Accordingly, an amino acid sequence is 50% "identical" to a second amino acid sequence when 50% of the amino acid residues of the two amino acid sequences are identical. The sequence comparison is performed over a contiguous block of amino acid residues comprised by a given protein, *e.g.*, a protein, or a portion of the polypeptide being compared. In a particular embodiment, selected deletions or insertions that could otherwise alter the correspondence between the two amino acid sequences are taken into account.

[0075] As used herein, DNA and protein sequence percent identity can be determined using C, MacVector (MacVector, Inc. Cary, NC 27519), Vector NTI (Informax, Inc. MD), Oxford Molecular Group PLC (1996) and the Clustal W algorithm with the alignment default parameters, and default parameters for identity. These commercially available programs can also be used to determine sequence similarity using the same or analogous default parameters. Alternatively, an Advanced Blast search under the default filter conditions can be used, *e.g.*, using the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin) pileup program using the default parameters.

[0076] As used herein a "nucleic acid" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. When referring to a nucleic acid that is double stranded both the "sense" strand and the complementary "antisense" strand are intended to be included. Thus a nucleic acid that is hybridizable to SEQ ID NOs: 1 or 3, for example, can be either hybridizable to the "sense" strand of the respective sequence, or to the "antisense" strand which can be readily determined from the respective sense strands listed in the Sequence Listing provided herein. The individual components of a nucleic acid are referred to as nucleotides.

[0077] A DNA "coding sequence" is a double-stranded DNA sequence that is transcribed and translated into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A nucleotide coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (*e.g.*, mammalian) DNA, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0078] Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

[0079] A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

[0080] A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which can then be trans-RNA spliced, if, when, and where appropriate, and translated into the protein encoded by the coding sequence.

[0081] A nucleotide sequence is "operatively linked" to an expression control sequence when the expression control sequence controls or regulates the transcription and translation of that nucleotide sequence. The term operatively linked includes having an appropriate start signal.

[0082] A "heterologous nucleotide sequence" as used herein is a nucleotide sequence that is added by recombinant methods to a nucleotide sequence encoding a polypeptide of the present invention or encoding a fragment thereof (*i.e.,* an antigenic fragment), to form a nucleic acid that is not naturally formed in nature. Such nucleic acids can *e.g.*, encode chimeric proteins. In addition, as used herein, a heterologous nucleotide sequence need not be a single contiguous nucleotide sequence, but can include multiple non-contiguous nucleotide sequences that have been combined with a nucleotide sequence encoding a polypeptide of the present invention, or a portion thereof. A heterologous nucleotide sequence can comprise non-coding sequences including restriction sites, regulatory sites, promoters and the like. In still another embodiment the heterologous nucleotide can function as a means of detecting a nucleic acid of the present invention.

[0083] The present invention provides heterologous nucleotide sequences that when combined with nucleotide sequences encoding a polypeptide of the invention or a fragment thereof, are necessary and sufficient to encode all of the chimeric proteins of the present invention. In a particular embodiment, the polypeptide comprises the amino acid sequence

of SEQ ID NO: 2.

[0084] As used herein, a bacterium is said to be "recombinant" when the nucleotide sequence of the DNA that it naturally contains has been purposely altered by at least one nucleotide addition, deletion, and/or modification through genetic engineering. A recombinant bacterin is an inactivated or killed recombinant bacterium.

[0085] The phrase "binding to" or "binds to" in regard to a ligand binding to a polypeptide (*e.g.*, antigen to an antibody) is used herein to include any or all such specific interactions that lead to a protein-ligand binding complex. This can include processes such as covalent, ionic (electrostatic and/or charged), hydrophobic and hydrogen bonding, but does not include non-specific associations such as solvent preferences.

[0086] As used herein a "small organic molecule" is an organic compound [or organic compound complexed with an inorganic compound (*e.g.*, metal)] that has a molecular weight of less than 3 kDa.

[0087] As used herein the terms "approximately" and "about" are used to signify that a value is within twenty percent of the indicated value *i.e.,* an amino acid sequence containing "approximately" 400 amino acid residues can contain between 320 and 480 amino acid residues.

[0088] As used herein the unit "° days" denotes the number of days of incubation following the vaccination of a fish, multiplied by the average temperature in °C for that incubation.

Nucleic Acids Encoding the Polypeptides of the Present Invention

[0089] A nucleic acid, such as a cDNA, that encodes a polypeptide of the present invention, can be placed into a vector, *e.g.*, a recombinant bacterial host cell, to express a protein and/or antigen of the present invention, *e.g.*, the $^{Ps}p_1$ 90 and $^{Ps}p_2$ 90 proteins. Such recombinant host cells can be inactivated, e.g., disrupted and converted to bacterins, and used in immunogenic compositions such as vaccines.

[0090] In addition, obtaining and/or constructing a DNA that encodes one of the polypeptides of the present invention, including those encoding $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90, or antigenic fragments thereof, facilitates the production of economically important quantities of the protein or antigenic fragments thereof. The large quantities of the proteins and/or antigenic fragments thereof produced are useful for making certain vaccines of the present invention.

[0091] Accordingly, the present invention provides specific nucleotide constructs that allow for the expression and isolation of large quantities of the proteins and/or antigens of the present invention, such as the $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90 proteins. These nucleic acids can further contain heterologous nucleotide sequences. To express a recombinant protein of the present invention in a host cell, an expression vector can be constructed comprising the corresponding cDNA. The present invention therefore, provides expression vectors containing nucleic acids encoding the proteins of the present invention, including variants thereof.

[0092] Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a nucleic acid encoding a polypeptide of the present invention may be used in the practice of the present invention. These include, but are not limited to, allelic genes, homologous genes from other strains, and/or those that are altered by the substitution of different codons that encode the same amino acid residue within the sequence, thus producing a silent change. Host cells comprising the expression vectors of the present invention are also provided. One particular host cell is an *E. coli* cell.

[0093] General methods for the cloning of cDNAs and expression of their corresponding recombinant proteins have been described [see Sambrook and Russell, Molecular Cloning, A laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor L.I. (2000)]. The particular methodology used herein is described in the Examples below. Preferably, all of the nucleic acid constructs of the present invention are sequence confirmed.

[0094] In addition, any technique for mutagenesis known in the art can be used to modify a native $^{Ps}p_1$ 90 or $^{Ps}p_2$ 90 protein of the present invention, including but not limited to, *in vitro* site-directed mutagenesis [Hutchinson et al., J. Biol. Chem., 253:6551 (1978); Zoller and Smith, DNA, 3:479-488 (1984); Oliphant et al., Gene, 44:177 (1986); Hutchinson et al., Proc. Natl. Acad. Sci. U.S.A., 83:710 (1986); Wang and Malcolm, BioTechniques 26:680-682 (1999). The use of TAB@ linkers (Pharmacia), etc. and PCR techniques also can be employed for site directed mutagenesis [*see* Higuchi, "Using PCR to Engineer DNA", in PCR Technology: Principles and Applications for DNA Amplification, H. Erlich, ed., Stockton Press, Chapter 6, pp. 61-70 (1989)].

[0095] The present invention also provides nucleic acids that hybridize to nucleic acids comprising the nucleotide sequences of the present invention. A nucleic acid is "hybridizable" to another nucleic acid, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid can anneal to the other nucleic acid under the appropriate conditions of temperature and solution ionic strength [see Sambrook and Russell, Molecular Cloning, A laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor L.I. (2000)].

[0096] The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleotides, low stringency hybridization conditions, corresponding to a T_m of 55°C, can be used, *e.g.,* 5X saline sodium citrate (SSC), 0.1% sodium dodecyl sufate (SDS), 0.25% milk, and no formamide; or 30% formamide, 5XSSC, 0.5% SDS. Moderate stringency hybridization conditions correspond to a higher T_m, *e.g.,* 40%

formamide, with 5X or 6XSSC. High stringency hybridization conditions correspond to the highest T_m, *e.g.,* 50% formamide, 5X or 6XSSC. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleotides having those sequences. The relative stability (corresponding to higher T_m) of nucleotide hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived [see Sambrook and Russell, Molecular Cloning, A laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor L.I. (2000)]. For hybridization with shorter nucleic acids, *i.e.,* oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity.

[0097] Depending upon circumstances a suitable minimal length for a hybridizable nucleic acid can be at least about 12 nucleotides; or at least about 18 nucleotides; or the length can be at least about 24 nucleotides; or at least about 36 nucleotides. Alternatively, the minimum length can be at least about 48 or at least about 72 nucleotides, or longer, as indicated above. In a specific embodiment, the term "standard hybridization conditions" refers to a T_m of 55°C, and utilizes conditions as set forth above. Under more stringent conditions, the T_m is 60°C, and under even more stringent conditions, the T_m is 65°C for both hybridization and wash conditions, respectively.

Polypeptides of the Present Invention

[0098] The present invention provides isolated and/or recombinant *Piscirickettsia salmonis* polypeptides, including all of the antigens of the present invention, *e.g.,* the $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90 proteins (plus or minus an amino-terminal signal peptide), *P. salmonis* strain variants thereof, antigenic fragments thereof, and chimeric proteins thereof. In addition, polypeptides containing altered sequences in which functionally equivalent amino acid residues are substituted for those within the wild type amino acid sequence resulting in a conservative amino acid substitution, are also provided by the present invention.

[0099] For example, one or more of these amino acid residues within the sequence can be substituted by another amino acid of a similar polarity, which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs.

[0100] For example, the nonpolar amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine and lysine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

[0101] Particularly preferred conserved amino acid exchanges are:

(a) Lys for Arg or *vice versa* such that a positive charge may be maintained;
(b) Glu for Asp or *vice versa* such that a negative charge may be maintained;
(c) Ser for Thr or *vice versa* such that a free -OH can be maintained;
(d) Gln for Asn or *vice versa* such that a free NH_2 can be maintained; and
(e) Ile for Leu or for Val or *vice versa* as being roughly equivalent hydrophobic amino acids.

[0102] All of the polypeptides of the present invention, including antigenic fragments, also can be part of a chimeric protein. In a specific embodiment, a chimeric polypeptide is expressed in a prokaryotic cell. Such a chimeric protein can be a fusion protein used to isolate a polypeptide of the present invention, through the use of an affinity column that is specific for a protein fused to the $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90 proteins, for example. Examples of such fusion proteins include: a glutathione-S-transferase (GST) fusion protein, a maltose-binding protein (MBP) fusion protein, a FLAG-tagged fusion protein, or a poly-histidine-tagged fusion protein. Specific linker sequences such as a Ser-Gly linker can also be part of such a fusion protein.

[0103] Indeed, the expression of one or more of the inventive proteins, as a fusion protein, can facilitate stable expression, and/or allow for purification based on the properties of the fusion partner. Thus the purification of the recombinant polypeptides of the present invention can be simplified through the use of fusion proteins having affinity Tags. For example, GST binds glutathione conjugated to a solid support matrix, MBP binds to a maltose matrix, and poly-histidine chelates to a Ni-chelation support matrix [see Hochuli et al., Biotechnology 6:1321-1325 (1998)].

[0104] The fusion protein can be eluted from the specific matrix with appropriate buffers, or by treating with a protease that is specific for a cleavage site that has been genetically engineered in between a $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90 protein, for example, and its fusion partner. Alternatively, a $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90 protein can be combined with a marker protein such as green fluorescent protein [Waldo et al., Nature Biotech. 17:691-695 (1999); U.S. Patent No. 5,625,048 and WO 97/26333].

[0105] Alternatively or in addition, other column chromatography steps (e.g., gel filtration, ion exchange, affinity chromatography etc.) can be used to purify the recombinant polypeptides of the present invention (see below). In many cases, such column chromatography steps employ high performance liquid chromatography or analogous methods in place of the more classical gravity-based procedures.

[0106] In addition, the polypeptides of the present invention, including the $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90 proteins, and antigenic fragments thereof, can be chemically synthesized [*see e.g.,* Synthetic Peptides: A User's Guide, W.H.Freeman & Co., New York, N.Y., pp. 382, Grant, ed. (1992)].

General Polypeptide Purification Procedures:

[0107] Generally, initial steps for purifying a polypeptide of the present invention can include salting in or salting out, in ammonium sulfate fractionations; solvent exclusion fractionations, e.g., an ethanol precipitation; detergent extractions to free membrane bound polypeptides, using such detergents as TRITON X- 100, TWEEN-20 etc.; or high salt extractions. Solubilization of membrane proteins may also be achieved using aprotic solvents such as dimethyl sulfoxide and hexamethylphosphoramide. In addition, high speed ultracentrifugation may be used either alone or in conjunction with other extraction techniques.

[0108] Generally good secondary isolation or purification steps include solid phase absorption using calcium phosphate gel, hydroxyapatite, or solid phase binding. Solid phase binding may be performed through ionic bonding, with either an anion exchanger, such as diethylaminoethyl (DEAE), or diethyl [2-hydroxypropyll aminoethyl (QAE) SEPHADEX or cellulose; or with a cation exchanger such as carboxymethyl (CM) or sulfopropyl (SP) SEPHADEX or cellulose. Alternative means of solid phase binding includes the exploitation of hydrophobic interactions e.g., the use of a solid support such as phenylSepharose and a high salt buffer; affinity-binding immuno-binding, using *e.g.*, a inventive protein bound to a suitable anti- $^{Ps}p_1$ 90 and/or anti-$^{Ps}p_2$ 90 selective antibody, respectfully, bound to an activated support. Other solid phase supports include those that contain specific dyes or lectins etc.

[0109] A further solid phase support technique that is often used at the end of the purification procedure relies on size exclusion, such as SEPHADEX and SEPHAROSE gels. Alternatively, a pressurized or centrifugal membrane technique, using size exclusion membrane filters may be employed. Oftentimes, these two methodologies are used in tandem.

[0110] Solid phase support separations are generally performed batch-wise with low-speed centrifugation, or by column chromatography. High performance liquid chromatography (HPLC), including such related techniques as FPLC, is presently the most common means of performing liquid chromatography. Size exclusion techniques may also be accomplished with the aid of low speed centrifugation. In addition size permeation techniques such as gel electrophoretic techniques may be employed. These techniques are generally performed in tubes, slabs or by capillary electrophoresis.

[0111] Almost all steps involving polypeptide purification employ a buffered solution. Unless otherwise specified, generally 25-100 mM concentrations of buffer salts are used. Low concentration buffers generally imply 5-25 mM concentrations. High concentration buffers generally imply concentrations of the buffering agent of between 0.1 - 2.0 M concentrations. Typical buffers can be purchased from most biochemical catalogues and include the classical buffers such as Tris, pyrophosphate, monophosphate and diphosphate and the Good buffers such as Mes, Hepes, Mops, Tricine and Ches [Good et al., Biochemistry, 5:467 (1966); Good and Izawa, Meth. Enzymo/., 24B:53 (1972); and Fergunson and Good, Anal. Biochem., 104:300 (1980].

[0112] Materials to perform all of these techniques are available from a variety of commercial sources such as Sigma Chemical Company in St. Louis, Missouri.

Antibodies to the Polypeptides of the Present Invention

[0113] The polypeptides of the present invention, and antigenic fragments thereof, as produced by a recombinant source, or through chemical synthesis, or as isolated from natural sources; and variants, derivatives or analogs thereof, including fusion proteins, may be used as an immunogen to generate antibodies. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric including single chain, Fab fragments, and a Fab expression library. Such antibodies can be used in diagnostic kits or as components in vaccines.

[0114] Specific anti- $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90 protein antibodies of the invention, for example, may be cross-reactive, that is, they may recognize one specific 90kDa protein, *e.g.*, $^{Ps}p_1$ 90, or a closely related protein obtained from a different source (*e.g.,* a *Piscirickettsia*-like bacterium). Polyclonal antibodies have greater likelihood of cross-reactivity. Alternatively, an antibody of the invention may be specific for a single form of an inventive protein, for example, such as a specific fragment of $^{Ps}p_1$ 90 that has the amino acid sequence of SEQ ID NO: 2, or a closely related variant thereof.

[0115] In a particular aspect of the present invention compositions and uses of antibodies that are immunoreactive with a $^{Ps}p_1$ 90 and optionally $^{Ps}p_2$ 90 protein are provided. Such antibodies "bind specifically" to the particular $^{Ps}p_1$ 90 and optionally $^{Ps}p_2$ 90 protein respectively, meaning that they bind *via* antigen-binding sites of the antibody as compared to non-specific binding interactions.

[0116] The terms "antibody" and "antibodies" are used herein in their broadest sense, and include, without limitation, intact monoclonal and polyclonal antibodies as well as fragments such as Fv, Fab, and F(ab') fragments, single-chain antibodies such as scFv, and various chain combinations. The antibodies may be prepared using a variety of well-known methods including, without limitation, immunization of animals having native or transgenic immune repertoires, phage display, hybridoma and recombinant cell culture.

[0117] Both polyclonal and monoclonal antibodies may be prepared by conventional techniques. [*See, for example,* Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Kennet et al. (eds.), Plenum Press, New York 37 (1980); and Antibodies: A Laboratory Manual, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988)].

[0118] Various procedures known in the art may be used for the production of polyclonal antibodies to a particular $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90 protein, variants or derivatives or analogs thereof. For the production of an antibody, various host animals can be immunized by injection with the $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90 protein, variant or a derivative (*e.g.*, or fusion protein) thereof or fragment thereof, including but not limited to rabbits, mice, rats, sheep, goats, etc. In one embodiment, the inventive protein can be conjugated to an immunogenic carrier, *e.g.*, bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, and dinitrophenol.

[0119] For preparation of monoclonal antibodies directed toward a given inventive protein, variant, or analog, or derivative thereof, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. These include but are not limited to the hybridoma technique originally developed by Kohler and Milstein [Nature, 256:495-497 (1975)], as well as the trioma technique, and the human B cell hybridoma technique [Kozbor et al., Immunology Today, 4:72 (1983); Cote et al., Proc. Natl. Acad Sci. U.S.A., 80:2026-2030 (1983)].

[0120] The monoclonal antibodies of the present invention include chimeric antibodies versions of antibodies originally produced in mice or other non-human animals. Techniques developed for the production of "chimeric antibodies" by splicing the genes from a mouse antibody molecule specific for a given inventive protein, for example, together with genes from a fish antibody of appropriate biological activity (*e.g.*, a salmon) can be used. Such chimeric antibodies are within the scope of this invention [*see in general,* Morrison et al., J Bacteriol, 159:870 (1984); Neuberger et al., Nature, 312:604-608 (1984); Takeda et al., Nature, 314:452-454 (1985)].

[0121] Hybridoma cell lines that produce monoclonal antibodies specific for the polypeptides of the present invention are also provided by the present invention. Such hybridomas may be produced and identified by conventional techniques.

[0122] One method for producing such a hybridoma cell line comprises immunizing an animal with a polypeptide, harvesting spleen cells from the immunized animal, fusing the spleen cells to a myeloma cell line, thereby generating hybridoma cells, and identifying a hybridoma cell line that produces a monoclonal antibody that binds the polypeptide. The monoclonal antibodies produced by hybridomas may be recovered by conventional techniques.

[0123] According to the invention, techniques described for the production of single chain antibodies [U.S. Patent Nos. 5,476,786, 5,132,405, and 4,946,778, can be adapted to produce *p. salmonis* protein-specific single chain antibodies, *e.g.,* $^{Ps}p_2$90 protein-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries [Huse et al., Science, 246:1275-1281 (1989)] to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90 protein, variant, derivative, and/or analog.

[0124] Antibody fragments which contain the idiotype of the antibody molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')2 fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

[0125] In the production of antibodies, screening for the desired antibody can be accomplished by such techniques as radioimmunoassay, enzyme-linked immunosorbant assay (ELISA), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays.

[0126] In one embodiment, antibody binding is detected by detecting a label, *e.g.*, a fluorescent label such as fluorescene isothiocyanate (FITC), on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention. For example, to select antibodies which recognize a specific epitope of a particular inventive protein, one may assay the hybridomas generated for a product which binds to a protein fragment containing such an

epitope and choose those which do not cross-react with a modified inventive protein that does not contain that epitope. One can select an antibody specific to $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90 from a particular source based on the positive specific binding with that specific protein.

SRS Vaccines

[0127] The present invention provides SRS vaccines. One particular embodiment is a non-mineral oil injection prime vaccine comprising one or more antigens from *Piscirickettsia salmonis,* as disclosed below. In one embodiment of this type, inactivated recombinant bacteria (bacterins) comprise one or more of the *Piscirickettsia salmonis* antigens of the present invention. The present invention also provides SRS vaccines that are designed to protect against one or more other fish pathogens. For example, furunculosis is an infectious ulcerative disease of salmon and trout caused by the bacterium *Aeromonas salmonicida.* In a particular embodiment, the vaccine will comprise in addition to the *Piscirickettsia salmonis* component(s), two Infectious *pancreatic necrosis* (IPN) antigens as discussed herein, *A. salmonicida* as discussed below, *Vibrio ordalii, Infectious Salmon Anemia,* and/or *Salmon Pancreatic Disease.*

[0128] Other fish pathogens include, but are not limited to:

PATHOGEN (antigen)	RELATED DISEASE
• IPN virus	Infectious pancreatic necrosis
• *Vibrio anguillarum* or *Vibrio ordalii*	Vibrosis
• *Vibrio salmonicida*	Cold water Vibriosis (Hitra disease)
• *Moritella viscosus*	Winter sores disease
• *Photobacterium damsela* (subspecies *Piscicida*)	Pasteurellosis
• *Lactococccus garviae Streptococcus iniae*	Streptococcosis
• *Moritella viscoses*	Winter Sores
• *Noccardia kampachi*	
• *Renibacterium salmoninarum*	
• *ISA Virus*	*Infectious Salmon Anemia*
• *IHN Virus*	Infectious Heamorhagic Necrosis
• *SPD Virus*	Salmon pancreatic disease
• *SD Virus*	Sleeping disease

[0129] The vaccines for these various diseases can be prepared from whole cells, bacterins, killed and/or attenuated virus, protein extracts, recombinant DNA vaccine vectors, isolated antigens, recombinant antigens and mixtures thereof. Under particular circumstances, as for *Photobacterium damsela* and *Aeromonas satmonicicla,* the vaccines are preferably prepared from two separate cultures grown under iron-depleted conditions and iron-supplemented conditions, respectively.

[0130] In a particular embodiment, a vaccine comprises the $^{Ps}p_1$ 90 and optionally $^{Ps}p_2$ 90 protein(s) from *Piscirickettsia salmonis.* In another embodiment, a vaccine comprises the $^{Ps}p_1$ 90 and optionally $^{Ps}p_2$ 90 protein(s) in combination with one or more of the above-noted other antigens from *Piscirickettsia salmonis* (*e.g.*,the ^{Ps}45 protein). In another embodiment, a vaccine comprises the $^{Ps}p_1$ 90 and optionally $^{Ps}p_2$ 90 protein(s), optionally in combination with *IPN* proteins, and optionally further in combination with one or more other antigens from *Piscirickettsia salmonis.* In still another embodiment, the vaccine comprises the $^{Ps}p_1$ 90 and optionally $^{Ps}p_2$ 90 protein(s) and/or one or more otherantigens from *Piscicketisia salmonis,* one or more IPN proteins, *and* one or more antigens to control *Aeromonas salmonicida.* In a particular embodiment of this type, *Aeromonas salmonicida* antigens are two types of whole bacteria grown on bacterial growth media and killed by the addition of formalin.

[0131] For an SRS vaccine according to the invention, *Escherichia coli* was selected as the best candidate for hosting and expressing the $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90 protein(s) of the present invention.

[0132] Two IPN viral antigens are exemplified below (*see also* WO 02/38770, the contents of which are hereby incorporated in its entireties). One of which is derived from Vp2, which is the major outer capsid protein and the other from Vp3, which is an internal protein of the IPN virus. The molecular weight of the Vp2 protein is 52 kDa, whereas that of the Vp3 protein is 30 kDa. The IPN proteins of the vaccines of the present invention are preferably purified recombinant proteins. In the Example 6 below, the IPN proteins are expressed and excreted by transformed yeast (*Pichia pastoris*) and then optionally purified from these yeast cells.

[0133] Antigens for a vaccine that also protects against furunculosis can be obtained from whole killed bacteria *Aeromonas* salmonicida (*e.g.*, formalin-killed). Early *A. salmonicida* vaccines contain whole *A. salmonicida* bacteria grown in normal growth medium and then inactivated by the addition of formalin. These bacterins contain a mixture of antigens

including the surface A-layer, inactivated proteases and lipopoly-saccharide. On the other hand when *A salmonicida* are grown in normal medium in the total absence of iron, a group of new antigens are expressed. These new antigens are termed iron-regulated outer membrane proteins (IROMP). IROMPS are highly immunogenic and they provide enhanced protection relative to vaccines containing inactivated *A. salmonicida* grown in normal medium. Four IROMP proteins having molecular weights of 82 kDa, 77 kDa, 72 kDa and 70 kDa respectively have been identified.

[0134] The primary and secondary antibody responses to IROMP antigens in Atlantic salmon (*Salmo salar*) immunized with A+ (iron *plus*) and A- (iron *minus*) *Aeromonas salmonicida* bacterins have been reported [O'Dowd et al., Fish & Shellfish Immunology 9:125-138 (1999)]. Thus particular vaccines of the present invention contain one strain of *A. salmonicida* (MT004) grown under conditions of iron-limitation and one strain of *A. salmonicida* (MT423) grown under condition of iron-supplementation.

[0135] The *Vibrio anguillarum* (serotype 01) and *V. anguillarum* (serotype 02) are different serotypes that are not cross-protective and therefore, for broad spectrum protection both antigens are can be included in the vaccine. Alternatively, or in combination, *Vibrio ordalii can* be employed.

Administration

[0136] The vaccines of the present invention may be administered to fish by any of a number of means including by injection (*e.g.*, intramucuscularly, or intraperitoneally), immersion, and/or through a delivery system for oral vaccination. Vaccinating fish by injection can be performed either with an adjuvant to increase the activity of the antigens, or without an adjuvant. Adjuvants include aqueous adjuvants, such as Alhydrogel or aluminum hydroxide, and oil adjuvants.

[0137] Mineral oil adjuvants are commonly employed in fish vaccines and are included in the present invention. One such adjuvant is mannide oleate in a mineral oil solution. In a particular embodiment of this type, the vaccine comprises 70% mannide oleate in a mineral oil solution. Another mineral oil adjuvant of the present invention consists of white mineral oil, Span 80 [sorbitan monooleate], and Tween 80 [polyoxyethylene sorbitan monooleate]. In a particular embodiment, a vaccine comprises 80% of an adjuvant having the following formulation: 944ml white mineral oil: 50.3ml Span 80: 5.7ml Tween 80.

[0138] Since mineral oil adjuvants generally cause damage to the fish at the site of injection (lesions, which have to be removed before sale) and they depress growth rates for a period of time, the present invention also provides non-mineral oil adjuvants. Synthetic non-mineral oil adjuvants include those commercially available from Seppic SA. Montanide, *e.g.,* Montanide ISA563, Montanide ISA 575, Montanide ISA 711, and Montanide ISA 760. Montanide ISA 711 is essentially mannide oleate in an oil solution. Particular embodiments of a vaccine of the present invention comprise 50% of either Montanide ISA563, Montanide ISA 575, Montanide ISA 760 or 70% Montanide ISA 711.

[0139] Alternatively, vaccines can be applied by a long-term immersion bath. In one such embodiment, vaccination *via* an immersion bath is preceded by hyperosmotic treatment [see Huising et al., Vaccine 21:4178-4193 (2003)]. In another embodiment, a vaccine is administered by spraying the fish.

[0140] The present invention also includes orally-delivered vaccines. Generally, oral vaccines are prepared by either top-dressing the food with an antigen (*e.g.,* by spray drying) or by incorporating the antigen in the food [*see, e.g.,* Vinitnantharat et al., Adv. Vet. Med. 41:539-550 (1999)]. Other techniques include water-in-oil methods, bioencapsulation, microencapsulation incorporation into liposomes, incorporation in hollow feed prills, and incorporation into microparticle carriers, *e.g.,* poly-lactide co-glycolide carrier particles [see, *e.g.,* Singh et al., Expert Opin. Biol. Ther. 4(4):483-491 (2004)]. Yet another method entails expressing the antigen in algae.

[0141] Booster vaccines are also part of the present invention. In a particular embodiment, an oily emulsion oral booster vaccine comprising one or more antigens from *Piscirickettsia salmonis* is used after the primary vaccination. Preferably the oily emulsion is made up of water:oil in the range of 6:4 to 4:6. The level of free fatty acids should not be greater than 5% by weight of the oil and preferably no greater than 3%. Particular oils include whole fish body oil and neutral marine oil. The emulsifier is preferably food grade. Lecithin can be used as such an emulsifier, *e.g.,* soya lecithin.

[0142] The emulsifier generally comprises from approximately 0.1% to approximately 5% by weight of the total emulsion. In a particular embodiment of this type, the oily phase of the emulsion is 47% v/v refined fish body oil *plus 3%* v/v lecithin (Bolec MT) which are mixed, sterilized with *gamma* irradiation and then blended, using an homogenizer. The aqueous antigen phase can be diluted with phosphate buffered saline [*see*, GB 2 255 909, PCT/GB9101828, WO/92/06599].

[0143] Injection vaccination is usually conducted on a commercial scale using a fixed dose automatic repeating syringe or an automatic injection vaccination machine. These methods are designed to deliver a fixed dose of usually 0.1 or 0.2 ml per fish. The vaccine is injected through the body wall into the intraperitoneal cavity. It is also possible to immunize fish by injecting the vaccine into the dorsal sinus. Generally, fish are vaccinated by injection following anesthetization.

[0144] Immersion vaccination can be performed as follows: Dilute 1 liter of vaccine with 9 liters of clean hatchery water. Then Drain and weigh a netful of fish and dip fish in the diluted vaccine for 30 to 60 seconds ensuring that fish are totally immersed in the vaccine. After 30 to 60 seconds lift net, drain and return fish to holding tank. Repeat until 100kg of fish

have been dipped into 10 liters of diluted vaccine.

[0145] Oral vaccination can be performed as follows: A container of vaccine is brought to room temperature (20°C) and then shaken prior to use. The vaccine is mixed with the fish feed so that the vaccine is coated onto the surface of the fish feed and adsorbed. The total vaccine dose should be fed over a 10 day period at 1/10 dose per fish per day.

VACCINATION RECIPIENTS

[0146] Salmonid rickettsial septicemia (SRS) was first observed in salmonids, which are the fish in the Salmonidae family, of the order Salmoniformes and of the class Osteichthyes. Salmonids are elongate bony fish with the last three vertebrae upturned, having a small adipose fin without fin rays between the dorsal fin and the tail. Many species of salmonids live in the sea, but enter fresh water to spawn. The Salmonidae family includes salmon, trout, char, and whitefish (see Table 1, below, which provides a non-exhaustive list of fish in the Salmonidae family).

TABLE 1

Salmonidae Family

Coregonus clupeaformis	Lake whitefish
Coregonus hoyi	Bloater
Oncorhynchus keta	Chum salmon
Oncorhynchus gorbuscha	Pink salmon
Oncorhynchus kisutch	Coho salmon (silver salmon)
Oncorhynchus masou	cherry salmon (masou salmon)
Oncorhynchus nerka	Sockeye salmon
Oncorhynchus tshawytscha	King salmon (chinook salmon)
Prosopium cylindraceum	Round whitefish
Oncorhynchus clarki	Cutthroat trout
Oncorhynchus mykiss	Rainbow trout
Salmo salar	Atlantic salmon
Salmo trutta	Brown trout
Salmo trutta X S. fontinalis	Tiger hybrid-trout
Salvelinus alpinus	Arctic charr
Salvelinus confluentus	Bull trout
Salvelinus fontinalis	Brook trout
Salvelinus leucomaenis	Japanese charr (white spotted charr)
Salvelinus malma	Dolly varden (Miyabe charr)
Salvelinus namaycush	Lake trout
Thymallus thymallus	Grayling

[0147] Reports of (SRS) and closely related Rickettsial syndrome afflicting fish as disparate as tilapia, white sea bass, rainbow trout, steelhead trout, grouper, Chilean sea bass, tiger puffers, red sea bream, blue-eyed plecostomus, striped bass, fluke, Atlantic cod, butter fish, ocean pout, spotted hake, summer and winter flounder, weakfish, yellowtail flounder, Windowpane flounder *(Scophthalmus aquosus)* cultured amberjack, three lined grunt, and blue eyed plecostomus indicates that the vaccines of the present invention may be used to vaccinate essentially any fish. Preferably the fish are in the *Teleosti* grouping of fish, *i.e.,* teleosts. Both the Salmoniformes order (which includes the Salmonidae family) and the Perciformes order (which includes the *Centrarchidae* family) are contained within the *Teleosti* grouping.

[0148] Aside from the *Salmonidae* family and those included above, examples of potential vaccination recipients include the *Serranidae* family, the *Sparidae* family, the *Cichlidae* family, the *Centrarchidae* family, the three-Line Grunt (*Parapristipoma trilineatum*), and the Blue-Eyed Plecostomus (*Plecostomus spp*) of Tables 2 and 3, below.

TABLE 2

Some Members of the *Serranidae* Family

TAXON NAME	COMMON NAME
Centropristis ocyurus	Bank sea bass
Centropristis philadelphicus	Rock sea bass
Centropristis striata	Black sea bass

(continued)

Some Members of the *Serranidae* Family

TAXON NAME	COMMON NAME
Diplectrum bivittatum	Dwarf sandperch
Diplectrum formosum	Sand perch
Epinephelus flavolimbatus	Yellowedge grouper
Epinephelus morio	Red grouper
Serranus phoebe	Tattler
Serranus tortugarum	Chalk bass

Some Members of the *Sparidae* family

TAXON NAME	COMMON NAME
Archosargus probatocephalus	Sheepshead
Archosargus rhomboidalis	Sea bream
Calamus penna	Sheepshead porgy
Lagodon rhomboides	Pinfish
Pagrus Major	Red Sea bream
Sparus aurata	Gilthead Sea bream
Stenotomus chrysops	Scup

Some Members of the *Cichlidae* family

TAXON NAME	COMMON NAME
Aequidens latifrons	Blue acara
Cichlisoma nigrofasciatum	Congo cichlid
Crenichichla sp.	Pike cichlid
Pterophyllum scalare	Angel fish
Tilapia mossambica	Mozambique mouth breeder
Oreochromis spp	Tilapia
Sarotherodon aurea	Golden Tilapia

TABLE 3

Some Members of the *Centrarchidae* family

TAXON NAME	COMMON NAME
Ambloplites rupestris	Rock bass
Centrarchus macropterus	Flier
Elassoma evergladei	Everglades pigmy sunfish
Elassoma okefenokee	Okefenokee pigmy sunfish
Elassoma zonatum	Banded pigmy sunfish
Enneacanthus gloriosus	Bluespotted sunfish
Enneacanthus obesus	Banded sunfish
Lepomis auritus	Redbreast sunfish
Lepomis cyanellus	Green sunfish
Lepomis cyanellus X L. gibbosus	Green x pumpkinseed
Lepomis gibbosus	Pumpkinseed
Lepomis gulosus	Warmouth
Lepomis humilis	Orange-spotted sunfish
Lepomis macrochirus	Bluegill
Lepomis megalotis	Longear sunfish
Micropterus coosae	Shoal bass
Micropterus dolomieui	Smallmouth bass

(continued)

Some Members of the *Centrarchidae* family

TAXON NAME	COMMON NAME
Micropterus punctulatus	Spotted bass
Micropterus salmoides	Largemouth bass
Pomoxis annularis	White crappie
Pomoxis nigromaculatus	Black crappie

[0149] The present invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention. The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLES

EXAMPLE 1

IDENTIFICATION Of TWO 90 kDA ANTIGENS From *PISCIRICKETTSIA SALMONIS*

[0150] Potential *Piscirickettsia salmonis* antigens were identified by screening a *P. salmonis* DNA expression library with polyclonal antibodies raised against *P. salmonis* as follows:

DNA was isolated from *P. salmonis* and partially digested with the restriction enzyme, *Sau*3AI. The isolated DNA was then cloned into the vector λGEM-12 (Promega) at a *Bam*HI site. λ phage structural proteins were then added and the phages were assembled. A phage library was produced containing 13,750 different phages. The library then was amplified in an *E. coli* host strain.

[0151] The library was next transferred to the pGEM-5zf (+) vector (Promega) *via* the *Not*I site. An *E. coli strain* was transformed with the library, grown in the presence of ampicillin, and selected for ampicillin resistance. Clones were screened by replica plating using nitrocellulose membranes. Following the lysis of the bacteria on the nitrocellulose membranes, the membranes were blocked with milk and then incubated with anti- *P. salmonis* polyclonal antibodies produced by immunizing rabbits with formaldehyde killed *P. salmonis* bacteria. Next, the membranes were washed and then developed with goat anti-rabbit-HRP conjugate. To detect recircularised plasmids that did not contain the inserts, the membranes were also exposed to X-gal. One isolated clone, designated 1057, appeared to express a 70-90 kDa protein. However, this putative 70-90 kDa protein was not purified, nor was it further characterized.

[0152] Initially, a Chilean strain of *Yersinia ruckeri,* a non-human enteric bacterium, was selected as the recombinant host cell to express the 70-90 kDa protein for possible use in a vaccine against SRS. This selection was based on the conventional wisdom that bacterial surface antigens are difficult to express in traditional bacterial vectors, particularly when the desired use for the recombinant vector would be as a vaccine antigen. Thus, it was believed the method of choice for expressing a *P. salmonis* surface antigen, as the 70-90 kDa protein was suspected of being, would be such a non-human enteric bacterium. Indeed, US Published Patent Application No. US20070207165 (A1) and WO2006037383(A1) specifically teach the use of such a recombinant *Yersinia ruckeri* vector to express *P. salmonis* proteins, and specifically exemplify the successful use of this vector to express the ^{Ps}p45 protein. In fact, this project was deemed completed when a recombinant *Yersinia* vector was isolated that was believed to encode the P. salmonis 70-90 kDa protein.

[0153] However, the isolated recombinant *Yersinia* vector was fraught with stability and expression problems. Indeed, it was never clear what this recombinant *Yersinia* vector expressed, and more importantly, never clear whether this recombinant *Yersinia* vector ever actually encoded a *P.* salmonis 70-90 kDa protein.

[0154] After considerable time and effort, this recombinant *Yersinia* vector was abandoned. The project was picked up again by sequencing the DNA of the parent clone 1057 clone. Surprisingly, rather than encoding one P. salmonis 70-90 kDa protein, it was found to contain 11 foreign open reading frames (ORFs). Furthermore, two of these open reading frames, ORF1 and ORF2, unexpectedly were found to encode approximately 90 kDa proteins. The sequences encoding these seemingly unrelated proteins were designated ORF1 (the $^{Ps}p_1$ 90 protein) and ORF2 (the $^{Ps}p_2$ 90 protein). The finding of 11 open reading frames, two of which encoded 90 kDa proteins, proves that the original assumption that the 1057 clone encoded a single *P. salmonis* antigen of about 90 kDa was incorrect.

[0155] The proteins encoded by ORF1 and ORF2 were further characterized. The protein encoded by ORF 1 ($^{Ps}p_1$

90) was found to have six predictive hydrophobic regions, whereas the protein encoded by ORF 2 ($^{Ps}p_2$ 90) was found to have only a single hydrophobic region.

[0156] The *P. salmonis* DNA sequence encoding $^{Ps}p_1$ 90 (ORF1).

SEQ ID NO: 1:

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ATGAAAAAGATAATTACAATGATGTTATTGGTGTTATCACTTGTGTTGGTCGCTTGTACC
CCAAGTGAAGAACCACCAACTACAGTGCCAGATGTTGAATCCATCGAATTTAATATGACT
TCAACGACTGTAGCACCAGGTGAACATACACTAGTTGCAAAAGCATTACCTGAAGGATCT
AATCAACAAATTAGATTTAGTATTCAAGGTATTGTATCTGGTGTATCCATTACGGGTGAT
AAGTTAAATGTTGGTAATGCTGTTGAAGATGGTATGAAATTTACAGTCGTAGCAACATCT
GTATATGATCCAACAATTCGTGCAACACTAGAGTTTACAGTTGTAAATGTTGGTGTTGAA
GTTGTTGAAATTAGAACAGAAGAAGAACTACGTGCAATTCATACAAATGAAGGTGGTTTA
TCATTATCTTATGTATTAATGAATGATATTGAACTAACAGCTCCATGGACACCAATTGGT
ATTGCTGAAGTTGAAACTGATTCTGGGCAAATCATTCCAGGTACGCCATTTAATGGTATC
TTTAATGGAAATGGTTTTACAATTAGTGGCATATTAGTTGAAAGTGAAGAACCATTATTT
AATGCAGGATTCTTTGCTCAAATTGGTGCAACTGCAATTGTTAAGAATACAACATTTGAA
GGTATAGTAAATGCAACCGGATGGTCCGGTGGTATCGCAGGTATTAACGAAGGTTTAATA
GAAAATGTTGTATCTAATGTAAGAGTTACTGTAACAGGTACATCCGCAGGTTCGCTAGTA
TCTGTTAATAGAGGTTTAATCCAATATGCTTACGGTATTGGTAAAGTTGTTAGTGAAACA
AACCCTAATACATCAGGTCGTTCTGCTGGTTTAGTGGTTGCTAATGATGGATCAATGATT
GAAGTGTATGGTGACTATCAAGCACTTGGAACACCTAACTATACAGCATTTAGTCCATCA
ACAAACCCAATGTATATGTTACCTACAGTAGATATGAAAACATCTTCAACTTGGGCTTCA
TTTGATGCAGATGTTTGGTATATTGAAAATGGTACATATCCATTATTAAAACATGAAGGA
TTCGTTCCACCAGTGATCGTTCCTGAATTAGGTATTACAATTAAAAATACTGAGTTAAAT
CATGATGTTGAAGTATCAAGTGAACTACAAATAAATGCAGAAGTCATTAACCCAGAAGGT
AGTGAAGTTATTGTTTATGCACTTAAAGAAGCAGTAGCAGGTGTAGCAATTAGTGAAACA
GGTTTAGTTACATTTGATATCACTACAATTGCTGCTAACTTCTCATTTACAGTAGTAGTG
ACAATTGATGGTACTGAAGTTAGTGCTGAAAAAACATTTACAGGCGTATATAACCCTGAA
ATTGTAGATGATACAGTGTATATTGAAACAGAAACACAATTATTAAACTTACTTGCTGGA
CAAACAAACCCAGACAATTTAAGTAAAACATTTGTATTATTAAATGATATTGTCTTAACT
TCTAATTGGACAGCAATTGGTATTGCACCAAATGAAGACGAAGGTATTGTAGGTGTTCCA
TTTACAGGTGTATTTGATGGTCAAGGCTATAAGATCTCAGGTATTAGTATGCCAGGTGGT
GGATGGAATAAAGGTTTCTTTGGATATATTGGAACAACTGGTGTTGTTAAAAACACACAC
TTTGAAGGTAATCTAGAAGCAAACGCATGGTCAGGTGCACTTGCAGCAAATAACTCAGGT
ACTATTCAAGATGTAGTTGTTGATATTGAAGTATATGTCTGGGGTAATAATGGTGGCGCA
ATCGTTGAACATAACCATGGTCTACTTAAAAATATTGTCGTATTAGGTAAAGCTGTATCA
GATAGTGGTCCTACAGCAGTTGGACTGGTTGTTACTAACTTTGGTACTTTAGAAAATGTA
TTTGCTAACGCAGATACAGTAGGTACAGCAAACTTAGTATCTAATGGTGCTCTCGCTGAT
GATGGTAAACACATTATTAGTGCCCAAGACTTTGTTAAAGCTACAACTTATGCAAACTTT
GATAGCGCAATCTGGTTAATCGTAGATGGCCAAGTACCTGTATTAATTAATGAAGATACA
GTATTACCTGAAACAGTAGTTTATATTGAAACAGAAGCAGAATTATTAAGCCTACTTGCT
GGTCAAGTAGATCCAGAAGCATTATCAAAAACATACAAACTTAAAAATGATATCGTTCTA
ACTTCTAATTGGACAGCAATTGGTATTGCACCAAATGAAGACGAAGGTATTGTAGGTGTT
CCATTTACAGGTGTATTTGATGGTCAAGGCTATAAGATCTCAGGTATTAGTATGCCAGGT
GGCGGATGGAATAAAGGTTTCTTTGGTTACATTGGAACAACGGGTGTTGTTAAAAATACA
CACTTTGAAGGTAATATTGAAGCAAACGCATGGTCAGGTGCACTTGCAGCAAATAACTCG
GGTACTATTATGGATGTTGTAGTAGACATTGAAGTATATGTCTGGGGTAATAATGGTGGT
GCAATTGTTGAACATAACCACGGTTTACTTAAAAATATTATCGTCTTAGGTAAAGCTGTA
TCAGATGGTGGTCCTACAGTAGTTGGACTAGTTGTTACTAACTTTGGTACACTAGAAGAT
GTATATGCAAATGTTGACACAGTAGGTACTTTAAACTTAGTATCATTTGGTAGCGTAGCA
GATGATGGTACACACATTATTAGTGCTTCAAACTTTGTTAAAGCAGAAACTTATGCAAAC
TTCTCAAGTGATGTTTGGACAATTATTGATGGTAGCACCCCTGTATTAAAACAAGCATAA
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[0157] The amino acid sequence of $^{Ps}p_1$ 90 (ORF1):

SEQ ID NO: 2

MKKIITMMLLVLSLVLVACTPSEEPPTTVPDVESIEFNMTSTTVAPGEHTLVAKALPEGS
NQQIRFSIQGIVSGVSITGDKLNVGNAVEDGMKFTVVATSVYDPTIRATLEFTVVNVGVE
VVEIRTEEELRAIHTNEGGLSLSYVLMNDIELTAPWTPIGIAEVETDSGQIIPGTPFNGI
FNGNGFTISGILVESEEPLFNAGFFAQIGATAIVKNTTFEGIVNATGWSGGIAGINEGLI
ENVVSNVRVTVTGTSAGSLVSVNRGLIQYAYGIGKVVSETNPNTSGRSAGLVVANDGSMI
EVYGDYQALGTPNYTAFSPSTNPMYMLPTVDMKTSSTWASFDADVWYIENGTYPLLKHEG
FVPPVIVPELGITIKNTELNHDVEVSSELQINAEVINPEGSEVIVYALKEAVAGVAISET
GLVTFDITTIAANFSFTVVVTIDGTEVSAEKTFTGVYNPEIVDDTVYIETETQLLNLLAG
QTNPDNLSKTFVLLNDIVLTSNWTAIGIAPNEDEGIVGVPFTGVFDGQGYKISGISMPGG
GWNKGFFGYIGTTGVVKNTHFEGNLEANAWSGALAANNSGTIQDVVVDIEVYVWGNNGGA
IVEHNHGLLKNIVVLGKAVSDSGPTAVGLVVTNFGTLENVFANADTVGTANLVSNGALAD
DGKHIISAQDFVKATTYANFDSAIWLIVDGQVPVLINEDTVLPETVVYIETEAELLSLLA
GQVDPEALSKTYKLKNDIVLTSNWTAIGIAPNEDEGIVGVPFTGVFDGQGYKISGISMPG
GGWNKGFFGYIGTTGVVKNTHFEGNIEANAWSGALAANNSGTIMDVVVDIEVYVWGNNGG
AIVEHNHGLLKNIIVLGKAVSDGGPTVVGLVVTNFGTLEDVYANVDTVGTLNLVSFGSVA
DDGTHIISASNFVKAETYANFSSDVWTIIDGSTPVLKQA

[0158] The *P. salmonis* DNA sequence encoding $^{Ps}p_2$90 (ORF2);

SEQ ID NO: 3:

ATGCATTTGGTGTTGTTGCTAAAAAGTAAAAAGGACAAATATATGAAAAAAATAAGC
TTAATAATGATTTTTCTGCTTTCTATCCTATTGGTAAGTTGTGTAGAAAAAGAAGAACCA
AAATTTGATCCAGATAAATATCTAGATTTAGAGAATATTGTATTTGATGATTTTGATAAC
GGAATTGACCCGAATATGTGGGTTATTGGTAATAGTAAGTGGGGTGTAGGTAATGGTGGT
GTCATCTATGAAAATGTCCATTACACAAATGACGGTATTGTAGTTCTTCAAACCAATGGT
GACTTGTATGATGGTCCACTTCGCGGTATTGGTAATACCCATGGCAGACGTACAGGTGCA
ATGATTACAACAAGAGAAGCACTAGGTCCTGGTAGATTTGAAGTACGTATGCGTATTATG
CCACGTTTTGGTTCAACTACTGCTATGTGGACTTACTATTATGATAATGGTATGAACCAT
GAAATAGATATCGAAAGTAACGTTGAAAATGACTTTAGAAAAGTATGGACTACAAACTGG
ATTAGTTTAACAGAATATAGTACTGTGTCTAATACCTTAGATTTTGCACAAAATGATTTT
GAATGGCGTACATACCGTTTTGACTGGTTTACAGATCCAAAACGCATTGATTATTATATT
GATGAAGTATTAGTTTCATCACAATCTTCTTATGTACCAGATCATGCAGGAGAATTTAAT
ATTGGTAATTGGTTTCCAGATGCTTGGGCAGGTGTACCTGATTTTGAAACAGACTATACC
TATGTAGACTGGTTCAAATATACACCATTTAAAGAACAACCATATACACCAACACCGGCA
AATAATCAAAGTCCTGCAAACTTCTATCCATCAGAACCAATTGAACATCCAATAGCAAAC
CTCATTTCAAATGCAGGTTTTGAAACAGATGCTCCAGCTTGGCGTTATCCTGTAACTAGT
GGTGTGGAACTACTAGAAGGTGAAGGTTTAAACGGATCAAGAGGAATCTTTGTTCCACAA
AATGATATTGCATATCAATTTGTCACAGGATTAGATGAAACCTTTGAAATGACATTTAGT
GCACATGCAAAACTACCTTTAAATGGTAGTGGATATGTTTTATTAGAGTTCTACCCAGCA
GAGACACAAAAAATTGATCAGTATATGATTGAGTTTAACTCAAGCGATGAAGATTTTATA
GCAGATACATTCTATGGTAAAGAATTTACCTTTAATGTACCTCTAGGAACTAAACGTGTT
GAAGTGTCTTTAATTGGAGGAGATTCTGGTATATACTTCGATGATTTATTCTTTAACCTA
ACTAAAAAACCCAGGCCCGAAATTGTAGAAGAAGGTGATGATGTGCAACGTTTAAACATA
GATTTTAAAAATGGTATTGACTCCAATGTTTGGGCAGTTGCAAATCAACGTTGGGGAGGT
ACACATCATGGTGGTGTAATCTTCCAAAACGTACACTACACAGAAGAAGGTAATTTACTC
ATTCAAGCCAATGGTGATTACTATGAAGGTCCATTAAAAGGTGTTGAACAAAATAATGGA
AAACGCACTGGGGGAGCTATCTATACTAAAGAAGCATTTGGCCCAGGATCTTTTGAAGTA
AAAGCTAAAATCATGCCACGTTTTGGGGCAACAACAGCATTTTGGACATTTAACTACTTA
GATGGTATTAATAGTGAAATTGATTTTGAGTTTAACGTAGGCAATGATTTTAGTACAGTT
TGGTTAACCAACTGGTTAACCGAAACAAACTATAACAACTACACCCATCAAATGGATAGT
TTCCATAATGATGGAAACTGGCATATATACCGTTTTGAATGGCATACACTACCGACGCCT
CATATTAAATACTTTATCGATGGCAAACTTGCATATACAGAACATACTAAAGTTCCAACG
ATGTCTGCAAGATACTGGATTGGTGTATGGTTTCCAAATAACTGGGCAGGAGATCCAAAC
TTTGAAACAGATTATTTAGAAGTTGAATATTTCAAATATGAGTCATTCCCGGATCATCCG
TATGTTGTTGGTCCAACTGGGGCATCCTCTCCAACAGCATTTTACCCAACAGCGCCAATA

AAAAAACCAGTTTCTAACCTTTTACCACACGGTAATCTAGATTATGAAACAGGTTATATG
TTAACAGGGGATGCAGTGATTTCAAATGGTGAATTGAAAACTGGTTTACTAGGCAGTGCT
GAGTCTCTTATTACAGGGTTAAATGATGCCTTTGAACTTACATTAAAGCTTAAAGCAAAA
GCCTCAAATAACGCAACCGTGCGCATTGAGTATTTAGATAAGGATTTAAATGTGATAAGT
GGTGAAGATATTATTGTATCAAACTTAAACGCGAATACATTTACAAACTTTACATCCGTA
ATTAATCTAGTGGAAGGCACTAGAGCCATCAATGTGATTTTTGAGGGAACAAATATCACA
TATGATGACTTATTTATAAATTTAACACACAAGGTGAATTGA

[0159] The amino acid sequence of $^{Ps}p_2$ 90 (ORF2):

SEQ ID NO: 4

MHLVLLLKSKKDKYMKKISLIMIFLLSILLVSCVEKEEPKFDPDKYLDLENIVFDDFDN
GIDPNMWVIGNSKWGVGNGGVIYENVHYTNDGIVVLQTNGDLYDGPLRGIGNTHGRRTGA
MITTREALGPGRFEVRMRIMPRFGSTTAMWTYYYDNGMNHEIDIESNVENDFRKVWTTNW
ISLTEYSTVSNTLDFAQNDFEWRTYRFDWFTDPKRIDYYIDEVLVSSQSSYVPDHAGEFN
IGNWFPDAWAGVPDFETDYTYVDWFKYTPFKEQPYTPTPANNQSPANFYPSEPIEHPIAN
LISNAGFETDAPAWRYPVTSGVELLEGEGLNGSRGIFVPQNDIAYQFVTGLDETFEMTFS
AHAKLPLNGSGYVLLEFYPAETQKIDQYMIEFNSSDEDFIADTFYGKEFTFNVPLGTKRV
EVSLIGGDSGIYFDDLFFNLTKKPRPEIVEEGDDVQRLNIDFKNGIDSNVWAVANQRWGG
THHGGVIFQNVHYTEEGNLLIQANGDYYEGPLKGVEQNNGKRTGGAIYTKEAFGPGSFEV
KAKIMPRFGATTAFWTFNYLDGINSEIDFEFNVGNDFSTVWLTNWLTETNYNNYTHQMDS
FHNDGNWHIYRFEWHTLPTPHIKYFIDGKLAYTEHTKVPTMSARYWIGVWFPNNWAGDPN
FETDYLEVEYFKYESFPDHPYVVGPTGASSPTAFYPTAPIKKPVSNLLPHGNLDYETGYM
LTGDAVISNGELKTGLLGSAESLITGLNDAFELTLKLKAKASNNATVRIEYLDKDLNVIS
GEDIIVSNLNANTFTNFTSVINLVEGTRAINVIFEGTNITYDDLFINLTHKVN

[0160] The DNA sequences encoding ORF 1 and ORF 2 were then optimized for *E. coli codon* usage, avoiding internal TATA boxes, chi sites and ribosomal entry sites, and AT or GC rich sequences. Synthetic genes were made and cloned via pPCR-Script into pEGT1 (the vector was constructed by EGT, and contains a T7/lac promoter, f1 origin, is Kanamycin resistant, (KanR), and Hok-Sok). Western blot analyses with anti-peptide antibodies to ORF 1 and ORF 2 confirmed expression of approximately 90 kDa proteins from both ORF 1 and ORF 2.

[0161] The *E.coli* optimized DNA sequence encoding $^{Ps}p_1$ 90 (ORF1); ORF1 sequences in pEGT1 (EGT optimised sequences):

SEQ ID NO: 5

ATGAAAAAAATCATCACCATGATGCTGCTGGTTCTGAGCCTGGTTCTGGTGGCGTGTACCCCGT
CTGAAGAACCGCCGACCACCGTTCCGGATGTGGAAAGCATTGAATTTAACATGACCAGCACCAC
CGTGGCACCGGGCGAACATACCCTGGTGGCGAAAGCGCTGCCGGAAGGCAGCAACCAGCAGATT
CGTTTTAGCATTCAGGGCATTGTGAGCGGCGTGAGCATTACCGGCGATAAACTGAACGTGGGCA
ACGCCGTGGAAGATGGCATGAAATTTACCGTTGTGGCGACCAGCGTGTATGACCCGACCATTCG
TGCCACCCTGGAATTTACCGTGGTTAACGTTGGCGTGGAAGTGGTGGAAATTCGTACCGAAGAA
GAACTGCGCGCGATTCATACCAACGAAGGCGGCCTGAGCCTGAGCTATGTGCTGATGAACGATA
TTGAACTGACCGCCCCGTGGACCCCGATTGGCATTGCCGAAGTGGAAACCGATAGCGGCCAGAT
TATTCCGGGCACCCCGTTTAACGGCATTTTTAACGGCAACGGCTTTACCATTAGCGGCATTCTG

GTGGAAAGCGAAGAACCGCTGTTTAACGCCGGCTTTTTTGCCCAGATTGGCGCCACCGCCATTG
TGAAAAACACCACCTTTGAAGGCATTGTGAACGCCACCGGCTGGAGCGGCGGCATTGCCGGCAT
TAACGAAGGCCTGATTGAAAACGTTGTTAGCAACGTTCGTGTGACCGTGACCGGCACCAGCGCC
GGTAGCCTGGTGAGCGTGAACCGTGGCCTGATTCAGTATGCCTATGGCATTGGCAAAGTGGTGA
GCGAAACCAACCCGAACACCAGCGGTCGTAGCGCCGGTCTGGTGGTGGCGAACGATGGCAGCAT
GATTGAAGTGTATGGCGATTATCAGGCGCTGGGCACCCCGAACTATACCGCCTTTAGCCCGAGC
ACCAACCCGATGTATATGCTGCCGACCGTGGATATGAAAACCAGCAGCACCTGGGCGAGCTTTG
ATGCCGATGTGTGGTATATCGAAAACGGCACCTATCCGCTGCTGAAACATGAAGGCTTTGTGCC
GCCGGTTATTGTGCCGGAACTGGGCATTACCATTAAAAACACCGAACTGAACCATGATGTGGAA
GTGAGCAGCGAACTGCAGATTAACGCCGAAGTGATTAACCCGGAAGGTAGCGAAGTTATTGTTT
ATGCCCTGAAAGAAGCGGTGGCGGGCGTTGCCATTAGCGAAACCGGCCTGGTGACCTTTGATAT
TACCACCATTGCGGCGAACTTTAGCTTTACCGTGGTGGTGACCATTGATGGCACCGAAGTGAGC
GCCGAAAAAACCTTTACCGGCGTGTATAACCCGGAAATTGTGGATGATACCGTCTATATCGAAA
CCGAAACCCAGCTGCTGAACCTGCTGGCGGGCCAGACCAACCCGGATAACCTGAGCAAAACCTT
TGTGCTGCTGAATGACATTGTGCTGACCAGCAACTGGACCGCCATTGGTATTGCCCCGAACGAA
GATGAAGGTATTGTTGGCGTTCCGTTTACCGGTGTGTTTGATGGCCAGGGCTACAAAATTAGCG
GTATTAGCATGCCGGGTGGCGGCTGGAACAAAGGCTTTTTTGGCTATATCGGCACCACCGGCGT
GGTGAAAAATACCCATTTCGAAGGTAACCTGGAAGCGAACGCCTGGTCTGGCGCCCTGGCGGCG
AACAACAGCGGCACCATTCAGGATGTGGTGGTGGATATCGAAGTGTATGTTTGGGGCAACAACG
GCGGTGCCATTGTGGAACATAACCATGGCCTGCTGAAAAACATTGTGGTGCTGGGTAAAGCGGT
GAGCGATAGCGGTCCGACCGCCGTGGGTCTGGTGGTTACCAACTTTGGCACCCTGGAAAACGTG
TTTGCCAACGCCGATACCGTGGGCACCGCCAACCTGGTGAGCAACGGTGCCCTGGCGGATGATG
GCAAACACATTATCAGCGCCCAGGATTTTGTGAAAGCGACCACCTATGCCAACTTTGATAGCGC
CATTTGGCTGATTGTGGATGGCCAGGTGCCGGTTCTGATTAACGAAGATACCGTGCTGCCGGAA
ACCGTGGTGTATATTGAAACCGAAGCGGAACTGCTGTCTCTGCTGGCGGGTCAGGTGGATCCGG
AAGCGCTGTCTAAAACCTACAAACTGAAAAACGATATCGTGCTGACCTCTAACTGGACGGCGAT
CGGCATCGCTCCGAATGAAGATGAGGGCATCGTCGGCGTCCCGTTCACCGGCGTGTTCGACGGT
CAGGGTTATAAAATTTCTGGCATTTCTATGCCGGGTGGTGGTTGGAATAAAGGTTTCTTCGGTT
ACATTGGCACCACCGGTGTTGTTAAAAACACTCACTTTGAGGGTAATATTGAAGCGAATGCCTG
GAGCGGCGCTCTGGCCGCCAACAACTCTGGCACCATTATGGATGTTGTTGTCGATATTGAAGTT
TACGTGTGGGGCAATAATGGTGGCGCCATCGTTGAACACAATCACGGTCTGCTGAAAAATATCA
TTGTTCTGGGTAAAGCCGTTTCTGATGGCGGTCCGACGGTGGTGGGCCTGGTTGTGACGAATTT
CGGCACGCTGGAAGATGTGTATGCCAATGTTGATACCGTTGGCACCCTGAATCTGGTGAGCTTT
GGCAGCGTGGCCGATGATGGCACCCATATCATTAGCGCCAGCAACTTTGTTAAAGCGGAAACCT
ATGCCAATTTTAGCAGCGATGTGTGGACCATTATTGATGGCAGCACCCCGGTGCTGAAACAGGC
GTAA

[0162] The *E.coli* optimized DNA sequence encoding $^{Ps}p_2$ 90 (ORF2); ORF2 sequences in pEGT1 (EGT optimised sequences). Note the corresponding *E.coli* optimized $^{Ps}p_2$ 90 amino acid sequence begins with an N-terminal histidine instead of the N-terminal proline for the naturally occurring $^{Ps}p_2$90 protein.

SEQ ID NO: 6

ATGCATCTGGTGCTGCTGCTGAAAAGCAAAAAAGATAAATACATGAAAAAAATCAG
CCTGATCATGATTTTTCTGCTGTCTATTCTGCTGGTGAGCTGTGTGGAAAAAGAAGAAC
CGAAATTCGATCCGGATAAATACCTGGATCTGGAAAACATCGTTTTCGATGATTTCGAT
AACGGCATTGATCCGAACATGTGGGTGATTGGCAACAGCAAATGGGGCGTGGGCAACGG
CGGCGTGATTTATGAAAACGTCCATTACACCAACGATGGCATTGTGGTGCTGCAGACCA
ACGGCGATCTGTATGATGGCCCGCTGCGTGGCATTGGCAACACCCATGGCCGTCGTACC
GGCGCCATGATTACCACCCGTGAAGCGCTGGGTCCGGGCCGTTTTGAAGTTCGTATGCG
CATTATGCCGCGTTTTGGCAGCACCACCGCCATGTGGACCTATTATTATGATAACGGCA
TGAACCACGAAATTGATATCGAAAGCAACGTGGAAAACGATTTTCGTAAAGTTTGGACC
ACCAACTGGATCAGCCTGACCGAATATAGCACCGTGAGCAACACCCTGGATTTTGCCCA
GAACGATTTTGAATGGCGTACCTATCGTTTTGATTGGTTTACCGATCCGAAACGTATCG

ATTACTACATTGATGAAGTGCTGGTGAGCAGCCAGAGCAGCTATGTGCCGGATCATGCC
GGCGAATTTAACATTGGCAACTGGTTTCCGGATGCCTGGGCAGGCGTTCCGGATTTTGA
AACCGATTATACCTACGTGGATTGGTTTAAATACACCCCGTTTAAAGAACAGCCGTATA
CCCCGACCCCGGCGAATAACCAGAGCCCGGCGAACTTTTATCCGAGCGAACCGATTGAA
CATCCGATTGCCAACCTGATTAGCAACGCCGGCTTCGAAACCGATGCCCCGGCATGGCG
TTATCCGGTGACCAGCGGCGTGGAACTGCTGGAAGGCGAAGGCCTGAACGGCAGCCGTG
GCATTTTTGTGCCGCAGAACGATATTGCCTATCAGTTTGTGACCGGCCTGGATGAAACC
TTTGAAATGACCTTTAGCGCCCATGCCAAACTGCCGCTGAACGGTAGCGGCTATGTGCT
GCTGGAATTTTATCCGGCGGAAACCCAGAAAATTGACCAGTATATGATCGAATTCAACA
GCAGCGATGAAGATTTTATCGCCGATACCTTCTATGGCAAAGAATTTACCTTTAACGTT
CCGCTGGGCACCAAACGTGTGGAAGTGAGCCTGATTGGCGGCGATAGCGGCATTTATTT
TGACGACCTGTTCTTCAACCTGACCAAAAAACCGCGTCCGGAAATTGTGGAAGAAGGCG
ACGACGTTCAGCGTCTGAACATTGATTTCAAAAACGGCATCGATAGCAACGTGTGGGCG
GTGGCGAATCAGCGTTGGGGCGGCACGCATCATGGCGGTGTGATTTTTCAGAACGTTCA
CTATACCGAAGAAGGCAACCTGCTGATTCAGGCGAACGGCGATTATTATGAAGGTCCGC
TGAAAGGCGTTGAACAGAACAACGGCAAACGTACCGGCGGTGCCATTTATACCAAAGAA
GCGTTTGGCCCGGGTAGCTTTGAAGTGAAAGCGAAAATCATGCCGCGCTTTGGTGCCAC
CACGGCGTTTTGGACCTTTAACTATCTGGATGGCATCAACAGCGAAATCGATTTTGAAT
TCAACGTGGGCAACGATTTTAGCACCGTGTGGCTGACCAACTGGCTGACCGAAACCAAC
TATAACAACTACACCCATCAGATGGATAGCTTTCATAACGATGGCAACTGGCATATTTA
TCGCTTTGAATGGCATACCCTGCCGACCCCGCATATTAAATACTTCATCGACGGCAAAC
TGGCGTATACCGAACATACCAAAGTGCCGACCATGAGCGCCCGTTATTGGATTGGCGTG
TGGTTTCCGAACAACTGGGCGGGTGATCCGAACTTTGAAACCGACTATCTGGAAGTGGA
ATACTTCAAATACGAAAGCTTTCCGGATCATCCGTATGTTGTTGGCCCGACCGGTGCCT
CTAGCCCGACCGCCTTTTATCCGACCGCCCCGATTAAAAAACCGGTGAGCAACCTGCTG
CCGCATGGCAACCTGGATTATGAAACCGGCTATATGCTGACCGGCGATGCCGTGATTAG
CAATGGCGAACTGAAAACCGGCCTGCTGGGCAGCGCCGAAAGCCTGATTACCGGCCTGA
ACGATGCCTTTGAACTGACCCTGAAACTGAAAGCGAAAGCGAGCAACAACGCCACCGTT
CGTATTGAATACCTGGATAAAGATCTGAACGTTATCAGCGGCGAAGATATTATTGTGAG
CAATCTGAACGCCAACACCTTTACCAACTTTACCAGCGTGATTAACCTGGTTGAAGGCA
CCCGTGCCATTAACGTTATTTTCGAAGGCACGAACATTACCTATGATGACCTGTTTATT
AACCTGACCCACAAAGTGAACTAATAAGTCGACG

EXAMPLE 2

EXPRESSION Of The TWO 90 kDA ANTIGENS From *PISCIRICKETTSIA SALMONIS*

Upstream Process

[0163] Batches of the two 90 kDa antigens were produced,

[0164] The strains used were as follows:

E. coli HMS174(DE3)/ pEGT1 /AL-ORF1- 90kDa ($^{Ps}p_1$ 90)
E. coli HMS174(DE3)/ pEGT1 /AL-ORF2- 90kDa ($^{Ps}p_2$ 90)

[0165] The strains were grown in shake-flasks. Each batch was prepared as a pool of 5 shake flasks.

The protocol was as follows:

[0166]

1. 50 µl of a glycerol stock was grown in 200mL of YES medium [30 g/l yeast extract, 5 g/l NaCl], supplemented with kanamycin 100 mg/l [500 ml medium in a 2 L shake-flask].
2. The culture was incubated at 37 °C, with an agitation of 270 rpm.
3. When the OD_{600} reached 2.1, protein expression was induced by the addition of 1 mM isopropyl-ß-D-thiogalact-opyranoside ("IPTG").
4. The final OD_{600} was around 2.2 for the ORF1-strain, and 1.7 for the ORF2-strain.
5. At the end of the culture, the pellets were harvested by centrifugation (5000 g, 30 min., 4 °C) and the supernatant

discarded.

6. Pellets were collected and stored at -20°C.

7. Cell breakage: Pellets were resuspended in 115 mL 20mM Tris buffer pH 7 for the ORF1-antigen and in 85 ml 20mM Tris buffer pH 7 for the ORF2-antigen. A French Press was used to break the cells and 2 cycles were performed (40K; 1000PSI; room temperature, all samples are keep on ice). Volumes collected were 120 ml for the ORF1-antigen and 75ml for the ORF2-antigen. The samples were stored at -20 °C.

EXAMPLE 3

Purfication of *PISCIRICKETTSIA SALMONIS* 90 kDa ANTIGENS

DOWNSTREAM PROCESS

[0167] 120 ml of the ORF1 antigen ($^{Ps}p_1$ 90) and 75 ml of the ORF2 ($^{Ps}p_2$ 90) antigen samples prepared according to Example 2 were semi-purified using a 300 kDa ultrafiltration. The retentates were concentrated to 50 ml and then diafiltered against 5 volumes of 20 mM Tris, pH 7.5. Both antigens were found to be in the retentate fractions forming large aggregates. The two antigens were then formulated by diafiltration with PBS buffer (137 mM NaCl, 2.7 mM KCl, 8 mM $Na_2HPO_4.12\ H_2O$, 1.5 mM KH_2PO_4, pH = 7.45) using a 300 kDa molecular weight cut-off ultrafiltration membrane.

EXAMPLE 4

Formulation Of Vaccines

[0168] 125 ml of the 300-kDa retentates, as produced by Example 3, were then diafiltrated against 5 volumes of PBS buffer (137 mM NaCl, 2.7 mM KCl, 8 mM $Na_2HPO_4.12\ H_2O$, 1.5 mM KH_2PO_4, pH = 7.45). The retentates containing the respective ORF-1 and ORF-2 90-kDa-antigens were collected and stored at - 20°C. The antigens were then sterile filtered through 0.2 μm filters prior to vaccine blending.

[0169] The final protein concentration for the two antigens preparations were 0.089 mg/ml for ORF1 and 0.015 mg/ml of ORF2. SDS PAGE gels were run and scanned in order to determine the ratio of the 90 kDa antigens to total protein. The ORF1 antigen was found to be 25.7 % of the total protein and the ORF 2 antigen was found to be 8.3 %. Final concentrations of the specific antigens were therefore 0.023 mg/ml for ORF 1 ($^{Ps}p_1$ 90) and 0.0012 mg/ml for ORF 2 ($^{Ps}p_2$ 90).

The vaccines were blended as follows:

[0170] The oil based adjuvant Montanide ISA 711 (Seppic) was used in a 70:30 (adjuvant:antigen) ratio.

Blend 1 (90 kDa ORF1)	15 ml (0.345 mg) antigen, 35 ml adjuvant
Blend 2 (90 kDa ORF2)	15 ml (0.018 mg) antigen, 35 ml adjuvant
Blend 3 (PBS control)	15 ml PBS, 35 ml adjuvant

Following mixing of the aqueous and oil phases, each of the respective vaccines was homogenised by serial passages through a syringe.

EXAMPLE 5

LABORATORY EFFICACY TRIAL Of VACCINATION Of ATLANTIC SALMON *(SALMO SALAR)* AGAINST SRS

[0171] The vaccines prepared according to Example 4 above, were tested for efficacy employing a challenge with *P. salmonis* at VESO Vikan, Norway.

[0172] Atlantic salmon (*Salmo salar* L., AquaGen Standard) were vaccinated at an average weight of 16.1 g in fresh water. Water temperature was 12 °C to 15 °C ± 1°C during the immunisation period and 15 °C ± 1°C during the *P. salmonis* challenges. The water flow rate was 0.8 l/ kg fish per min. The fish density was a maximum of 40 kg/m^3.

[0173] Fish were acclimatised for 18 days after arrival at VESO Vikan. 105 fish were anesthetized and injected with 0.1 ml of each vaccine per fish, and a control group of 105 fish was anesthetized and injected with 0.1 ml of physiological saline with 0.9% NaCl per fish. The fish were tagged with ink and by fin cutting. The test groups and the control group were pooled after vaccination. 12 days before challenge the fish were sorted into two parallel tanks, with 50 fish per

group per tank. 10 days before challenge the water temperature was raised to 15°C.

Laboratory efficacy trial - challenge

[0174] 48-52 fish from each group were challenged with *P. salmonis* by intraperitoneal injection in two duplicate tanks 8 weeks after vaccination. The same challenge dose was used in both tanks (0.1 ml 1:1000 dilution per fish, and the dose was determined following prechallenge experiments). The temperature in the rearing water was 15°C during the challenge. The challenge material was grown at The National Veterinary Institute, Oslo and was kept at -75°C until challenge. On the day of challenge, the challenge material was dose adjusted according to the results obtained in the pre-challenge. The challenge material was diluted in PBS with 1.3 % NaCl.

[0175] Relative percentage survival (RPS_{60}) was calculated on the day that control group mortality equalled exactly 60 % or, if inappropriate, at the time corresponding to 60 % control group mortality (t60) according to the expression:

$$RPS_{60} = (1-(\frac{MV_{60}x100}{n_v \times 60})) \times 100$$

[0176] In cases where control-group mortality did not equal exactly 60% on any given day of the trial, cumulative mortality of vaccinates at the time corresponding to 60 % control group mortality was estimated from the expression.

$$MV_{60} = MV_{60-1} + (\frac{((0.6 \times n_c) - MC_{60-1}) \times (MV_{60+1}-MV_{60-1})}{(MC_{60+1} - MC_{60-1})})$$

[0177] The following definitions apply to the parameters of the first and second expressions, above.

MV_{60} = cumulative mortality of vaccinates at 60% cumulative control group mortality.
MC_{60-1} = number of mortalities in control group on last day before cumulative mortality rate in control group exceeds 60%.
MC_{60+1} = number of mortalities in control group on first day after cumulative mortality rate in control group has exceeded 60%.
MV_{60-1} = number of mortalities in vaccinated group on last day before cumulative mortality rate in control group exceeds 60%.
MV_{60+1} = number of mortalities in vaccinated group on first day after cumulative mortality rate in control group has exceeded 60%.
n_c = total number of fish in control group.
n_v = total number of fish in vaccinated group
RPS_{60} = Relative percentage survival at 60% control group mortality.

[0178] RPS_{60} was subsequently calculated from the first expression, with the following results.

Results

[0179] Results for the challenge are summarized by FIG. 1, and by the tables below. As can be seen in FIG. 1, the challenged animals vaccinated by saline control (diamonds) or adjuvant control (squares) exhibited up to about 75% mortality by days 25-30 post challenge. In contrast, animals vaccinated with the ORF-1 antigen ($^{Ps}p_1$ 90), denoted by "X" and the ORF-2 antigen ($^{Ps}p_2$90) denoted by triangles, exhibited both delayed and reduced mortality. In particular, animals vaccinated with ORF-2 ($^{Ps}p_2$90) exhibited no more than 5% mortality out to day 32.

[0180] Cumulative mortality as defined by RPS_{60}

TABLE 4

Tanks A and B were set up with a random mix of vaccinated and saline vaccinated fish.			
Vaccine	**RPS_{60} Tank A**	**RPS_{60} Tank B**	**Mean RPS_{60}**
90 kDa ORF 1 ($^{Ps}p_1$ 90)	96.8	100.0	**98.4**
90 kDa ORF 2 ($^{Ps}p_2$ 90)	50.0	77.1	**63.6**

(continued)

Tanks A and B were set up with a random mix of vaccinated and saline vaccinated fish.			
Vaccine	**RPS_{60} Tank A**	**RPS_{60} Tank B**	**Mean RPS_{60}**
Adjuvant + PBS	2.0	11.7	**6.9**

[0181] In order for the test results to be acceptable, mortalities in the control group must reach 60 % within 30 days after the first specific mortality has been recorded. As the results met these criteria the results were accepted as valid.

[0182] The results therefore demonstrate that both the 90 kDa ORF 1 antigen ($^{Ps}p_1$ 90) and the 90 kDa ORF 2 antigen ($^{Ps}p_2$90) are capable to provide significant protection in salmon against SRS caused by *P. salmonis.*

EXAMPLE 6

Production Method Of VP2var or VP3 (50 Liter Scale)

[0183] *The yeast strains: Pichia pastoris* GS115 - pPICZαB - VP2var. The *Pichia* expression system is used to express the IPN protein antigens [Research Corporation Technologies, Tucson, Arizona, see U.S. Patent Nos. 4,808,537, 4,837,148, 4,879,231, the contents of which are hereby incorporated by reference in their entireties.

[0184] *Preculture:* A 2-liter baffled shake-flask containing 400 ml of YSG+ (see below) is inoculated with 600 μl of the above-identified yeast strain. The culture is incubated at 30 °C, with an agitation of 270 rpm, during 23 - 25 hours. The optical density at 600nm (OD_{600nm}) is >15 units (using a NOVASPEC II spectrophotometer), as set forth by Table 5, below.

TABLE 5

Composition of the Medium YSG+:	
COMPONENTS	CONCENTRATION
Yeast Extract	6 g/l
Papaïc Soy Pepton	5 g/l
Glycerol	20 g/l

[0185] *Fermentation:* The fermentor Braun D50 is prepared with 50 liters of growth medium (SAPPEY, see below). The fermentor is inoculated with a volume ($V_{preculture}$) of preculture determined by the equation:

- $$V_{preculture}\ (ml) = V_{fermentor}\ (ml) \times 0.05 / OD_{600preculture}$$

Where, $V_{fermentor}$ is defined as the volume of the growth medium in the fermentor, and the $OD_{600preculture}$ is the optical density determined at 600 nm of the preculture solution obtained above.

TABLE 6

Parameters for Fermentation	
PARAMETERS	SET POINTS
pH	6*
Temperature	30°C
Air-flow	80 l/min.
PO_2	30% **
Agitation	400-(600) rpm

(continued)

Parameters for Fermentation	
PARAMETERS	SET POINTS
Pressure	100 mbar
* regulation with acid (HNO_3 10 %) and base (NH_4OH 12.5%) ** with an action on the agitation to maintain the PO_2 at 30% Automatic regulation of foam with SAG471.	

TABLE 7

Composition of Growth Medium SAPPEY per 1 Liter:	
Components	Volumes
Base solution	900 ml
Complement solution 1	100 ml
PTM1 solution	4.76 ml

TABLE 8

Quantities per 1 Liter of Base Solution [The solution is autoclaved in the fermentor (20 min., 121°C)]	
Components	Quantity
Yeast Extract	11.11 g/l
Papaïc Soy Pepton	22.22 g/l
Antifoam SAG471	0.11 ml/l

TABLE 9

Quantities per 1 Liter of COMPLEMENT SOLUTION 1 (The solution is sterilised by filtration with a 0.22μm pore membrane)	
Components	Quantity
K_2HPO_4	23 g/l
KH_2PO_4	118 g/l
Glycerol	100 g/l

TABLE 10

Quantity for 1 Liter of PTM1 SOLUTION	
Components	Quantity
$CuSO_4.5H_2O$	6 g/l
NaI	0.08 g/l
$MnSO_4.H_2O$	3 g/l
$Na_2MoO_4.2H_2O$	0.2 g/l

(continued)

Quantity for 1 Liter of PTM1 SOLUTION	
Components	Quantity
H_3BO_3	0.02 g/l
$CoCl_2.6H_2O$	0.92 g/l
$ZnCl_2$	20 g/l
$FeSO_4.7H_2O$	65 g/l
d-biotine	0.2 g/l
H_2SO_4	5 ml/l

[0186] The solution is sterilized by filtration with a 0.22μm pore membrane. The PTM1 solution must be added in the fermentor separately from the complement solution 1.

TABLE 11

Composition of "INDUCTION SOLUTIONS" per 1 Liter (The methanol is added by sterile filtration with a 0.22μm pore membrane)		
	Components	Volumes
	Methanol 100 %	6.3 ml/l of culture
	Yeast Extract solution	22.5 ml/l of culture

TABLE 12

Quantity for 1 Liter of YEAST EXTRACT SOLUTION [This solution is autoclaved (20 min., 121°C)]	
Components	Quantity
Yeast Extract	222 g/l

[0187] After 24 hours of growth, a first induction of recombinant protein expression is realized by the addition of methanol and yeast extract solution. At this moment, the OD_{600}nm is greater than about 10 units. After the induction the pO_2 decreases quickly. After about 1 hour, it increases slowly to saturation. A second induction is realized after 48 hours of culture in the same conditions. The OD_{600nm} reached is greater than about 13 units. After 72 hours of growth, the fermentor is cooled to a temperature lower than 20°C. The OD_{600nm} reached is greater than about 13 units.

[0188] *Harvest and filling:* The cells from the fermentor are then harvested. The culture is centrifuged (5000g, 4°C, 20 min) in order to eliminate the pellets. The supernatant is aseptically filtrated with a 0.2 μm pore membrane (Sartobran P) and 2.5 liter aliquots are placed into one gallon bottles. These bottles are then stored at -20°C.

EXAMPLE 7

AN INJECTABLE VACCINE FOR SRS, IPN AND FURUNCULOSIS

Summary

[0189] One injectable vaccine of the present invention is a water-in-oil type vaccine that comprises a suspension of:

(i) two inactivated strains of *Aeromonas salmonicida* (MT004 and MT423),
(ii) two recombinant IPN viral proteins (VP2 and VP3) or antigenic fragments thereof, that are expressed by transformed yeast, *Pichia pastoris* in 0.85% p/v sterile saline, and
(iii) a suspension comprising inactivated recombinant strains of *E.coli* encoding $^{Ps}p_1$ 90 and/or $^{Ps}p_2$ 90, in phosphate buffered saline.

[0190] The VP2 (VP2var) recombinant proteins are expressed by transformed yeast, *Pichia pastoris* BCCM Accession No. IHEM 20069 and/or BCCM Accession No. IHEM 20070, whereas the VP3 recombinant proteins are expressed by BCCM Accession No. IHEM 20071, and/or BCCM Accession No. IHEM 20072. The oily adjuvant is MONTANIDE ISA711 and constitutes 70% of the vaccine's total volume. The formulation may contain residual amounts of formaldehyde, derived from inactivation of the cultures.

[0191] This particular vaccine is designed and recommended for administration by intraperitoneal injection, to protect against salmonid rickettsial septicaemia, infectious pancreatic necrosis and furunculosis in fish, more particularly salmonids, and even more particularly, in salmon.

Presentation

[0192] This vaccine is presented in 500 ml high density polyethylene infusion flasks, closed with grey nitrile stoppers and having aluminium seals. The bottles and stoppers comply with the requirements of the relevant monographs of the European Pharmacopoeia (Ph. Eur). The containers are autoclaved at 121° C for 20 minutes. The stoppers are autoclaved at 121° C for 60 minutes.

Production

Production of A. salmonicida MT004 Antigen:

[0193] An ampoule of lyophilized work seed is removed from storage and is reconstituted and incubated. This culture is then inoculated in 4 liters of sterile iron-deficient TSB to form the production culture, and then incubated at approximately 21.5° C for 36-48 hours.

[0194] The resulting culture is then aseptically inoculated in 15-18 liters of sterile iron-deficient TSB. It is incubated at approximately 21.5 °C for 24 to 48 hours. Then a solution of sterile formaldehyde is added to the flasks to inactivate the culture. Each culture is mixed vigorously following the addition of the formaldehyde solution and is then transferred aseptically to a sterile storage bottle. The culture is kept at approximately 22° C for 96-100 hours to ensure the inactivation of bacterial cultures and protease activity. The formaldehyde is neutralized by the addition of a solution of 15% sodium metabisulfite. Neutralisation is completed in 20-24 hours at a temperature of approximately 22° C. The inactivated harvests are stored at 2-8° C until they are required for mixing. The production *of A. salmonicida* MT004 antigen can also be performed as described below for MT423.

Production of A. salmonicida MT423 Antigen:

[0195] An ampoule of lyophilized work seed is removed from storage and reconstituted and incubated. This culture is then inoculated in 300 ml of sterile iron-supplemented TSB to form the production culture, and then incubated at approximately 21.5° C for 36-48 hours.

[0196] The culture is next inoculated aseptically in 4 liters of sterile iron-supplemented TSB. It is incubated at approximately 21.5° C for 36 to 48 hours. The culture of production seed is transferred aseptically to 150 liters of sterile iron-supplemented TSB in a fermentor and incubated at approximately 21.5° C for 20-24 hours.

[0197] Then a solution of sterile formaldehyde is added to the culture flasks to inactivate them. Each culture is mixed vigorously following the addition of the formaldehyde solution and is transferred aseptically to a sterile storage bottle. The culture is kept at approximately 22° C for 96-100 hours to ensure inactivation of the bacterial cultures and protease activity. The formaldehyde is neutralized by adding a solution of 15% sodium metabisulfite. Neutralization is completed in 20-24 hours at a temperature of approximately 22° C. The inactivated harvests are stored at 2-8° C until they are required for mixing

Production of Recombinant Proteins IPN (VP2 VAR) and IPN VP3:

[0198] Recombinant proteins IPN (VP2 VAR) and IPN VP3 are prepared and stored as described in Example 6 above.

Production of Antigens of Piscirickettsia salmonis:

[0199] The $^{Ps}p_1$ 90 and $^{Ps}p_2$ 90 antigens are prepared for the formulation of the vaccine as described in Example 4 above.

Mixing of the Final Vaccine

[0200] Bulk antigens are mixed with the other antigen components, phosphate-buffered saline solution, and the oil component to obtain a bulk vaccine of the desired cell concentration.

[0201] The volumes of bulk antigens required (calculated on the individual concentrations of bulk antigen, the required concentrations of these in the end product and the batch size) are removed from storage. The bulk antigens are transferred to cool, sterile containers and are mixed thoroughly.

[0202] The volume of sterile saline required is calculated and transferred aseptically to the mixed bulk antigens. The antigens and saline are thoroughly mixed and the pH is adjusted to pH 7.0 - 7.4 with 10 M sodium hydroxide or 10 M hydrochloric acid (aqueous phase).

[0203] The weight of sterile oily phase required is calculated and transferred aseptically to a sterile mixing container. The oily and aqueous phases are emulsified for 5 minutes at approximately 3000 rpm. The emulsified mix is maintained at ambient temperature for 24 hours. The mix is placed in the final containers, with a nominal fill value of 505 ml. The stoppers are inserted aseptically and the seals are applied. Each container is labeled, packaged and stored at +2° C to +8° C under quarantine until released for sale. The batch size varies according to production requirements and is normally within the range of 100 liters to 1500 liters.

Materials

[0204] Piscirickettsia salmonis antigens are prepared as described above. In addition two strains of *Aeromonas salmonicida* are used, which derive from isolated naturally infected fish obtained from fish farmed in Scotland.
In spite of the fact that there is no evidence that there is any serological distinction between different strains of *Aeromonas salmonicida,* there is a scientific basis for including more than one strain in this vaccine. This is due to the fact that different isolated ones may be A-layer positive or negative. Considering that the presence or absence of this layer may not be directly linked to virulence, the absence of an A-layer allows greater exposure to outer membrane proteins (OMPs), and in particular, those OMPs produced only under conditions of iron restriction, as may occur during the infection process. As a result, the production and immunological availability of the iron restriction outer membrane proteins (IROMPs) is thought to be important to the efficacy of the vaccine.

Aeromonas salmonicida (MT004):

[0205] The MT004 strain is an A-layer negative strain which is cultivated under conditions of iron restriction. Development under these conditions results in the production of specific iron restriction outer membrane proteins that stimulate the production of bacterial antibodies following intraperitoneal inoculation.

[0206] The strain was originally isolated from dying Atlantic salmon during an outbreak of furunculosis in on a salmon farm on the West Coast of Scotland in October 1985. It was passaged through tryptone soya broth six times and remained virulent to the host animal.

Aeromonas salmonicida (MT423):

[0207] The MT423 strain is an A-layer positive strain that has been cultivated in a fermentor under conditions of iron restriction. A-layer is a component of successful *A. salmonicida* vaccines and supplementation with iron has increased the protection afforded by the furunculosis vaccine.

[0208] The MT423 strain was isolated from sick Atlantic salmon from a salmon farm at Stirling University. It was passaged 16 times in Atlantic salmon and remained virulent to the host animal and is therefore appropriate for use as a vaccine strain.

[0209] Both strains are inactivated by exposure to formaldehyde, being in non-infecting organisms, whereas it retains its ability to stimulate an immune response in vaccinated fish.

[0210] The vaccine also contains the recombinant proteins VP2 var and VP3 of IPNV as described in Example 6 above.

[0211] Other Reagents are provided in Table 13:

TABLE 13

Reagents		
REAGENT	COMPONENTS	CHARACTERISTICS
Tryptone Soya Broth (TSB)	Pancreatic casein digestive enzyme	Cow's milk from herds certified BSE free, originally from France, but currently from New Zealand. Porcine enzymes from France, Italy and Holland.
	Soya digestive papain	No materials of biological origin
	Sodium chloride	
	Hydrogenated dipotassium phosphate	
	Dextrose	Synthetic or of non-animal origin
	Purified water	Meets the requirements of the European Pharmacopoeia.
Hydrochloric acid (pH adjustment)	-	Meets the requirements of the European Pharmacopoeia.
Sodium hydroxide (pH adjustment)	-	Meets the requirements of the European Pharmacopoeia.
Formaldehyde (Inactivator)	-	Meets the requirements of the European Pharmacopoeia.
Saline solution (Diluent)	Sodium chloride	Meets the requirements of the European Pharmacopoeia.
	Purified water	Meets the requirements of the European Pharmacopoeia.
Montanide ISA711 (Adjuvant)	Contains oleic acid	EDQM Certified available

Assays

[0212] Several tests are carried out to ensure that the consistency and quality of the vaccine and its components are maintained. These tests are described below.

Aeromonas salmonicida Strains MT004 and MT423:

[0213] The test methods used for both antigens are the same, except that the test for the presence of IROMPS is not used for the MT423 strain, since this is multiplied in an iron-enriched medium. In addition, the criteria used for some tests are different for each strain. For the sake of simplicity, the following test descriptions specify the criterion for each strain where it is appropriate.

Purity Tests - Gram Stain:

[0214] Gram stain purity tests are carried out on each subculture during multiplication from seed to production culture. The test provides a rapid indication that the cultivated organism has the hoped for microscopic appearance and that no atypical organism is present.

[0215] The test method is a simple Gram stain that uses conventional techniques and materials. Known Gram positive and negative control organisms are stained each time to confirm that staining and discoloration are appropriate. The test sample must only show small Gram negative rods.

Purity Test and Characteristics of the Culture:

[0216] An additional purity test is carried out on each of the 20 liter complete cultures and on the culture in the final fermentor. The test confirms the purity of the culture and contributes to global identity security. A sample of the culture is grown on plates of tryptone soya agar and incubated at 22° C for at least 48 hours, long enough for the different colonies to become visible. Plates inoculated with the test culture must exhibit only one type of bacterial colony. These

colonies must be typical of *Aeromonas salmonicida.*

[0217] The *Aeromonas salmonicida* MT004 strain forms semi-translucent, round, convex, cream-colored colonies with regular edges. A red-brown pigmentation is produced which spreads through the medium after around 24 hours of culture. The *Aeromonas salmonicida* MT423 strain: Semi-translucent, round, convex, cream-colored colonies with regular edges, but developing more slowly than the MT004 strain.

[0218] *Identity of the culture:* The identity of a given culture is confirmed in the samples on final fermentation. Identity tests are carried out on the final culture prior to inactivation to confirm that the correct organism has been cultured. It must be emphasized that none of these tests can differentiate the strains, but all contribute to the security of identifying the species. In addition to the purity tests, identity is confirmed by means of biochemical and agglutination characteristics:

- Demonstration of the use of glucose without gas production.
  A sample from the final culture is inoculated in peptone water containing 1% glucose and phenol red in tubes containing an inverted Durham tube. The inoculated cultures are incubated at 22° C for 24-48 hours. The test sample must show the use of glucose, indicated by acid production, without gas being produced.

- *Demonstration of positive metabolism of cytochrome oxidase using commercially available impregnated filter papers:*
  A single colony from the purity test plate (culture characteristics) is spread over the filter paper. A positive result is indicated by the development of a pinkish purple pigment while a negative result is indicated by no color change. The cultures must generate a pinkish purple coloration on the test paper, indicating positive cytochrome oxidase metabolism.

- *Lattes cover-glass test using a diagnostic kit of pathogens from commercial fish (Bionor MONO-AS - Code DD020).*
  A single colony from the purity test plate (culture characteristics) is mixed with a drop of antiserum on a microscope slide. The test uses a specific rabbit antiserum against *Aeromonas salmonicida.* A negative control culture is likewise mixed with a drop of antiserum. Positive agglutination must be observed with the test sample. The negative control sample must not show any agglutination.

Optical Density:

[0219] Optical density measurements at 580 nm are recorded at the end of each culture in 20 liter bottles and at intervals throughout final fermentation. Optical density measurements are taken from 20 liter culture bottles to ensure that each of these inoculants has grown satisfactorily. Optical density measurements are recorded at intervals throughout final fermentation to determine the optimum time for harvest, as indicated at the end of the exponential growth phase.

[0220] A sample of the culture is placed in a cuvette and the optical density is measured directly using a spectrophotometer. If necessary, the sample may be diluted in 0.85% sterile saline solution in order to obtain opacity within the spectrophotometer's range. The method is only used to confirm satisfactory growth of the inoculant and to determine the optimum time for harvest of the final fermentation. The final optical density reading is not critical and no set criterion applies. However, the final value obtained from the culture in the fermentor is normally within the following range:

- 8 - 11 for MT004 strain (without iron)
- 13-18 for MT423 strain (iron supplemented)

[0221] The absolute criterion for optical density is not appropriate for several reasons. First, considering that the medium used is of biological origin, there is inevitably a variation in the degree to which a specific batch will support growth. Second, the frequency of sampling for optical density is restricted to 45 minute intervals due to the need to re-sterilize the sampling port. Consequently, the precise harvest time may allow the culture to be maintained in the stationary phase for a short period of time, during which a reduction in optical density may be observed.

Viable Count

[0222] A sample of the culture is taken for the viable count at the end of fermentation and prior to adding the inactivator. The viable count serves as a definitive measurement of yield and forms the basis for subsequent mixing of the vaccine. The viable count is carried out using the Miles and Misra method *[see e.g.,* Hedges, *Int J Food Microbiol.* **25**:76(3):207-14 (2002)] with Tryptone Soya Broth as diluent and Tryptone Soya Agar as growth medium. Suitable ten-fold serial dilutions of the sample are prepared and ten replicate 0.025ml drops of each dilution placed on the agar plate. The plates are incubated at 22° C for 24-48 hours. Only those dilutions where colonies may be clearly counted are used to calculate the viable count.

[0223] The viable count is used as the basis for mixing the vaccine. The actual count is not critical and no set criterion

is applied. However, normal counts are within the range 0.3 - 1.5 x 10^{10}/ml for both strains MT004 and MT423. The absolute criterion is not appropriate for several reasons. First, considering that the medium used is of biological origin, there is inevitably a variation in the degree to which a specific batch will support growth.

[0224] Second, the frequency of sampling for optical density is restricted to 45 minute intervals due to the need to re-sterilize the sampling port. Consequently, the precise harvest time may allow the culture to be maintained in the stationary phase for a short period of time, during which a reduction in optical density may be observed.

Protease Test

[0225] The protease test is carried out on a sample of material taken immediately following the inactivation period, but before the addition of sodium thiosulphate. With the improved control of the culture's conditions, no release of protease has been observed. However, because it is possible to sample the culture from the final fermentor at intervals of no less than 45 minutes, there is the possibility that some cells will die, and consequently lysis may occur prior to inactivation. This test provides the reassurance that any protease that may be released is completely inactivated.

Protease Assay:

[0226] 3 ml of inactivated culture is added to 20mg of SKY BLUE powder suspended in 2.5 ml of PBS and incubated for 15 minutes at ambient temperature. A positive control in which 20 mg of trypsin replaces the test samples is also incubated. The SKY BLUE powder is insoluble in PBS, but if protease activity is present, the material degrades and blue dye is released into the solution. The positive control must show a blue color while negative controls must remain colorless. To be acceptable, the test samples must not exhibit any protease activity. Positive samples must show a blue coloration.

Inactivation Test

[0227] A specific test for inactivation of the culture is carried out following neutralization of the residual inactivator. A subsequent test for continuous and complete inactivation is carried out on the mixed aqueous phase of the vaccine. The test confirms the complete, satisfactory inactivation of all viable organisms.

Inactivation assay:

[0228] 1 ml of inactivated culture is inoculated in each of six tubes containing 9 ml of TSB. Two of these inoculated tubes are inoculated with 0.1 ml of positive control culture with *Aeromonas salmonicida* of the same strains as the sample being tested, inoculating with a designated concentration of between 1 and 10 organisms. Two further inoculated tubes are additionally inoculated with 0.1 ml using the same positive control culture diluted 1 in 10. Also 0.1 ml of both positive control preparations are inoculated in two tubes, each containing 9.9 ml of TSB and another two tubes of TSB medium are kept only as negative controls. Therefore, duplicates of the following tubes are prepared (a total of 12 tubes in all):

- Inoculated with 1 ml of test sample
- Inoculated with 1 ml of test sample + 0.1 ml positive control
- Inoculated with 1 ml of test sample + 0.1 ml positive control (diluted 1/10)
- Inoculated with 0.1 ml positive control
- Inoculated with 0.1 ml positive control diluted 1/10
- Not inoculated

[0229] All of the above tubes are incubated for 48 hours at 22° C. At the end of this time, any tube in which growth cannot be seen is subcultivated. Subcultivation is carried out by spreading 1 ml of the medium onto each of two plates of tryptone soya agar. The medium is left to absorb into the agar for 1 hour at ambient temperature and the plates are incubated (inverted) for 48 hours at 22° C. The original tubes are also incubated for 48 hours at 22° C.

[0230] At the end of this time, growth (or absence of growth) is recorded in all cultures. The criterion of being acceptable is that all the tubes inoculated with the test sample only and all plates inoculated from these must not show any growth. In addition, all tubes inoculated with the highest concentration of organisms of the positive control and/or all plates inoculated from these must show growth of the control organism. If the tubes inoculated exclusively with the lowest dilution of the positive control culture and/or the plates inoculated from these show growth, similar results must be observed for the tubes and plates inoculated with the test sample plus the diluted positive control. The control mediums must remain negative.

Test for IROMPS

[0231] This test only applies to the material of strain MT004 and applies to a sample of final bulk antigen following inactivation and neutralization but, prior to distributing the material between the storage containers. The test is a qualitative method for confirming the presence of typical iron-restricted proteins in the preparation.

[0232] SDS-PAGE electrophoresis is performed on the sample. The SDS-PAGE gels are electroblotted to PVDF membranes that are then incubated with a rat monoclonal antibody against IROMP. Coupling of the monoclonal antibody is detected by a conjugate of goat anti-rat alkaline phosphatase and displayed using a NBT-BCIP substrate. A positive control preparation of *Aeromonas salmonicida* IROMP is spread on the same gel together with the molecular weight markers. The method is qualitative, but the acceptance criterion requires that the samples exhibit bands consistent with those of the control preparation. More particularly, protein bands must be detected at about 70, 72, 77 and 82 kilodaltons.

Steril ity

[0233] The sterility of each container of final bulk antigen is confirmed using a specific sterility test although the inactivation test also provides additional evidence of sterility of the bulk product prior to distribution. The test provides the assurance that each container of bulk antigen is sterile.

[0234] The method used is that indicated in the Ph. Eur. Using direct inoculation thioglycollate and soya broths are incubated at 32° C and 22° C respectively, and both are subcultivated after 14 days of incubation. The subcultures are incubated for 7 days, while the original cultures are incubated for a total of 21 days. The method includes positive control cultures specified in the Ph. Eur.

[0235] To be acceptable the samples being tested must be sterile. The positive control cultures must show profuse early growth (within 3 days).

TABLE 14

SEQUENCES	
SEQ ID NO.:	DESCRIPTION
1	Nucleotide sequence encoding the genomic 1057 ORF 1 gene.
2	Amino acid sequence expressed by the ORF 1 gene [$^{Ps}p_1$90].
3	Nucleotide sequence encoding the genomic 1057 ORF 2 gene.
4	Amino acid sequence expressed by the ORF 2 gene [$^{Ps}p_2$90].
5	Nucleotide sequence encoding the PEGT1 ORF 1 gene.
6	Nucleotide sequence encoding the PEGT1 ORF 2 gene.
7	Amino acid sequence of the 45 kDa protein.
8	Amino acid sequence of the 45 kDa protein *minus* the signal peptide.
9	Amino acid sequence of an AMP binding enzyme homolog.
10	Amino acid sequence of ORF A.
11	Amino acid sequence of ORF B.
12	Amino acid sequence of a DDE endonuclease homolog.
13	Amino acid sequence of a transposase homolog.
14	Amino acid sequence of an HlyD homolog.
15	Amino acid sequence of an AcrB/AcrD/AcrF homolog.
16	2,092 nucleotide nucleotide sequence comprising the coding sequence of the 45 kDa protein.

SEQUENCE LISTING

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Kirke, David F.
Francis, Michael J.

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        115                 120                 125

Glu Leu Arg Ala Ile His Thr Asn Glu Gly Gly Leu Ser Leu Ser Tyr
    130                 135                 140

Val Leu Met Asn Asp Ile Glu Leu Thr Ala Pro Trp Thr Pro Ile Gly
145                 150                 155                 160

Ile Ala Glu Val Glu Thr Asp Ser Gly Gln Ile Ile Pro Gly Thr Pro
                165                 170                 175

Phe Asn Gly Ile Phe Asn Gly Asn Gly Phe Thr Ile Ser Gly Ile Leu
            180                 185                 190

Val Glu Ser Glu Glu Pro Leu Phe Asn Ala Gly Phe Phe Ala Gln Ile
        195                 200                 205

Gly Ala Thr Ala Ile Val Lys Asn Thr Thr Phe Glu Gly Ile Val Asn
    210                 215                 220

Ala Thr Gly Trp Ser Gly Gly Ile Ala Gly Ile Asn Glu Gly Leu Ile
225                 230                 235                 240
```

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Glu Asn Val Val Ser Asn Val Arg Val Thr Val Thr Gly Thr Ser Ala
               245                 250                 255

Gly Ser Leu Val Ser Val Asn Arg Gly Leu Ile Gln Tyr Ala Tyr Gly
           260                 265                 270

Ile Gly Lys Val Val Ser Glu Thr Asn Pro Asn Thr Ser Gly Arg Ser
       275                 280                 285

Ala Gly Leu Val Val Ala Asn Asp Gly Ser Met Ile Glu Val Tyr Gly
   290                 295                 300

Asp Tyr Gln Ala Leu Gly Thr Pro Asn Tyr Thr Ala Phe Ser Pro Ser
305                 310                 315                 320

Thr Asn Pro Met Tyr Met Leu Pro Thr Val Asp Met Lys Thr Ser Ser
               325                 330                 335

Thr Trp Ala Ser Phe Asp Ala Asp Val Trp Tyr Ile Glu Asn Gly Thr
           340                 345                 350

Tyr Pro Leu Leu Lys His Glu Gly Phe Val Pro Pro Val Ile Val Pro
       355                 360                 365

Glu Leu Gly Ile Thr Ile Lys Asn Thr Glu Leu Asn His Asp Val Glu
   370                 375                 380

Val Ser Ser Glu Leu Gln Ile Asn Ala Glu Val Ile Asn Pro Glu Gly
385                 390                 395                 400

Ser Glu Val Ile Val Tyr Ala Leu Lys Glu Ala Val Ala Gly Val Ala
               405                 410                 415

Ile Ser Glu Thr Gly Leu Val Thr Phe Asp Ile Thr Thr Ile Ala Ala
           420                 425                 430

Asn Phe Ser Phe Thr Val Val Val Thr Ile Asp Gly Thr Glu Val Ser
       435                 440                 445

Ala Glu Lys Thr Phe Thr Gly Val Tyr Asn Pro Glu Ile Val Asp Asp
   450                 455                 460

Thr Val Tyr Ile Glu Thr Glu Thr Gln Leu Leu Asn Leu Leu Ala Gly
465                 470                 475                 480
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Gln Thr Asn Pro Asp Asn Leu Ser Lys Thr Phe Val Leu Leu Asn Asp
                485                 490                 495

Ile Val Leu Thr Ser Asn Trp Thr Ala Ile Gly Ile Ala Pro Asn Glu
            500                 505                 510

Asp Glu Gly Ile Val Gly Val Pro Phe Thr Gly Val Phe Asp Gly Gln
        515                 520                 525

Gly Tyr Lys Ile Ser Gly Ile Ser Met Pro Gly Gly Gly Trp Asn Lys
    530                 535                 540

Gly Phe Phe Gly Tyr Ile Gly Thr Thr Gly Val Val Lys Asn Thr His
545                 550                 555                 560

Phe Glu Gly Asn Leu Glu Ala Asn Ala Trp Ser Gly Ala Leu Ala Ala
                565                 570                 575

Asn Asn Ser Gly Thr Ile Gln Asp Val Val Val Asp Ile Glu Val Tyr
            580                 585                 590

Val Trp Gly Asn Asn Gly Gly Ala Ile Val Glu His Asn His Gly Leu
        595                 600                 605

Leu Lys Asn Ile Val Val Leu Gly Lys Ala Val Ser Asp Ser Gly Pro
    610                 615                 620

Thr Ala Val Gly Leu Val Val Thr Asn Phe Gly Thr Leu Glu Asn Val
625                 630                 635                 640

Phe Ala Asn Ala Asp Thr Val Gly Thr Ala Asn Leu Val Ser Asn Gly
                645                 650                 655

Ala Leu Ala Asp Asp Gly Lys His Ile Ile Ser Ala Gln Asp Phe Val
            660                 665                 670

Lys Ala Thr Thr Tyr Ala Asn Phe Asp Ser Ala Ile Trp Leu Ile Val
        675                 680                 685

Asp Gly Gln Val Pro Val Leu Ile Asn Glu Asp Thr Val Leu Pro Glu
    690                 695                 700

Thr Val Val Tyr Ile Glu Thr Glu Ala Glu Leu Leu Ser Leu Leu Ala
705                 710                 715                 720
```

```
Gly Gln Val Asp Pro Glu Ala Leu Ser Lys Thr Tyr Lys Leu Lys Asn
                725                 730                 735

Asp Ile Val Leu Thr Ser Asn Trp Thr Ala Ile Gly Ile Ala Pro Asn
            740                 745                 750

Glu Asp Glu Gly Ile Val Gly Val Pro Phe Thr Gly Val Phe Asp Gly
        755                 760                 765

Gln Gly Tyr Lys Ile Ser Gly Ile Ser Met Pro Gly Gly Gly Trp Asn
    770                 775                 780

Lys Gly Phe Phe Gly Tyr Ile Gly Thr Thr Gly Val Val Lys Asn Thr
785                 790                 795                 800

His Phe Glu Gly Asn Ile Glu Ala Asn Ala Trp Ser Gly Ala Leu Ala
                805                 810                 815

Ala Asn Asn Ser Gly Thr Ile Met Asp Val Val Val Asp Ile Glu Val
            820                 825                 830

Tyr Val Trp Gly Asn Asn Gly Gly Ala Ile Val Glu His Asn His Gly
        835                 840                 845

Leu Leu Lys Asn Ile Ile Val Leu Gly Lys Ala Val Ser Asp Gly Gly
    850                 855                 860

Pro Thr Val Val Gly Leu Val Val Thr Asn Phe Gly Thr Leu Glu Asp
865                 870                 875                 880

Val Tyr Ala Asn Val Asp Thr Val Gly Thr Leu Asn Leu Val Ser Phe
                885                 890                 895

Gly Ser Val Ala Asp Asp Gly Thr His Ile Ile Ser Ala Ser Asn Phe
            900                 905                 910

Val Lys Ala Glu Thr Tyr Ala Asn Phe Ser Ser Asp Val Trp Thr Ile
        915                 920                 925

Ile Asp Gly Ser Thr Pro Val Leu Lys Gln Ala
    930                 935
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<212> DNA
<213> Piscirickettsia salmonis

<400> 3

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tttgatccag ataaatatct agatttagag aatattgtat ttgatgattt tgataacgga 180

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atctatgaaa atgtccatta cacaaatgac ggtattgtag ttcttcaaac caatggtgac 300

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attacaacaa gagaagcact aggtcctggt agatttgaag tacgtatgcg tattatgcca 420

cgttttggtt caactactgc tatgtggact tactattatg ataatggtat gaaccatgaa 480

atagatatcg aaagtaacgt tgaaaatgac tttagaaaag tatggactac aaactggatt 540

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tggcgtacat accgttttga ctggtttaca gatccaaaac gcattgatta ttatattgat 660

gaagtattag tttcatcaca atcttcttat gtaccagatc atgcaggaga atttaatatt 720

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aatcaaagtc ctgcaaactt ctatccatca gaaccaattg aacatccaat agcaaacctc 900

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tcaaataacg caaccgtgcg cattgagtat ttagataagg atttaaatgt gataagtggt 2340

gaagatatta ttgtatcaaa cttaaacgcg aatacattta caaactttac atccgtaatt 2400

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<212> PRT
<213> Piscirickettsia salmonis

<400> 4

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Lys Ile Ser Leu Ile Met Ile Phe Leu Leu Ser Ile Leu Leu Val Ser
20 25 30

Cys Val Glu Lys Glu Glu Pro Lys Phe Asp Pro Asp Lys Tyr Leu Asp
35 40 45

Leu Glu Asn Ile Val Phe Asp Asp Phe Asp Asn Gly Ile Asp Pro Asn
50 55 60

Met Trp Val Ile Gly Asn Ser Lys Trp Gly Val Gly Asn Gly Gly Val
65 70 75 80

Ile Tyr Glu Asn Val His Tyr Thr Asn Asp Gly Ile Val Val Leu Gln
85 90 95

Thr Asn Gly Asp Leu Tyr Asp Gly Pro Leu Arg Gly Ile Gly Asn Thr
100 105 110

His Gly Arg Arg Thr Gly Ala Met Ile Thr Thr Arg Glu Ala Leu Gly

```
        115                 120                 125

Pro Gly Arg Phe Glu Val Arg Met Arg Ile Met Pro Arg Phe Gly Ser
    130                 135                 140

Thr Thr Ala Met Trp Thr Tyr Tyr Tyr Asp Asn Gly Met Asn His Glu
145                 150                 155                 160

Ile Asp Ile Glu Ser Asn Val Glu Asn Asp Phe Arg Lys Val Trp Thr
                165                 170                 175

Thr Asn Trp Ile Ser Leu Thr Glu Tyr Ser Thr Val Ser Asn Thr Leu
            180                 185                 190

Asp Phe Ala Gln Asn Asp Phe Glu Trp Arg Thr Tyr Arg Phe Asp Trp
        195                 200                 205

Phe Thr Asp Pro Lys Arg Ile Asp Tyr Tyr Ile Asp Glu Val Leu Val
    210                 215                 220

Ser Ser Gln Ser Ser Tyr Val Pro Asp His Ala Gly Glu Phe Asn Ile
225                 230                 235                 240

Gly Asn Trp Phe Pro Asp Ala Trp Ala Gly Val Pro Asp Phe Glu Thr
                245                 250                 255

Asp Tyr Thr Tyr Val Asp Trp Phe Lys Tyr Thr Pro Phe Lys Glu Gln
            260                 265                 270

Pro Tyr Thr Pro Thr Pro Ala Asn Asn Gln Ser Pro Ala Asn Phe Tyr
        275                 280                 285

Pro Ser Glu Pro Ile Glu His Pro Ile Ala Asn Leu Ile Ser Asn Ala
    290                 295                 300

Gly Phe Glu Thr Asp Ala Pro Ala Trp Arg Tyr Pro Val Thr Ser Gly
305                 310                 315                 320

Val Glu Leu Leu Glu Gly Glu Gly Leu Asn Gly Ser Arg Gly Ile Phe
                325                 330                 335

Val Pro Gln Asn Asp Ile Ala Tyr Gln Phe Val Thr Gly Leu Asp Glu
            340                 345                 350

Thr Phe Glu Met Thr Phe Ser Ala His Ala Lys Leu Pro Leu Asn Gly
        355                 360                 365
```

```
Ser Gly Tyr Val Leu Leu Glu Phe Tyr Pro Ala Glu Thr Gln Lys Ile
    370                 375                 380

Asp Gln Tyr Met Ile Glu Phe Asn Ser Ser Asp Glu Asp Phe Ile Ala
385                 390                 395                 400

Asp Thr Phe Tyr Gly Lys Glu Phe Thr Phe Asn Val Pro Leu Gly Thr
                405                 410                 415

Lys Arg Val Glu Val Ser Leu Ile Gly Gly Asp Ser Gly Ile Tyr Phe
            420                 425                 430

Asp Asp Leu Phe Phe Asn Leu Thr Lys Lys Pro Arg Pro Glu Ile Val
        435                 440                 445

Glu Glu Gly Asp Asp Val Gln Arg Leu Asn Ile Asp Phe Lys Asn Gly
    450                 455                 460

Ile Asp Ser Asn Val Trp Ala Val Ala Asn Gln Arg Trp Gly Gly Thr
465                 470                 475                 480

His His Gly Gly Val Ile Phe Gln Asn Val His Tyr Thr Glu Glu Gly
                485                 490                 495

Asn Leu Leu Ile Gln Ala Asn Gly Asp Tyr Tyr Glu Gly Pro Leu Lys
            500                 505                 510

Gly Val Glu Gln Asn Asn Gly Lys Arg Thr Gly Gly Ala Ile Tyr Thr
        515                 520                 525

Lys Glu Ala Phe Gly Pro Gly Ser Phe Glu Val Lys Ala Lys Ile Met
    530                 535                 540

Pro Arg Phe Gly Ala Thr Thr Ala Phe Trp Thr Phe Asn Tyr Leu Asp
545                 550                 555                 560

Gly Ile Asn Ser Glu Ile Asp Phe Glu Phe Asn Val Gly Asn Asp Phe
                565                 570                 575

Ser Thr Val Trp Leu Thr Asn Trp Leu Thr Glu Thr Asn Tyr Asn Asn
            580                 585                 590

Tyr Thr His Gln Met Asp Ser Phe His Asn Asp Gly Asn Trp His Ile
        595                 600                 605
```

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Tyr Arg Phe Glu Trp His Thr Leu Pro Thr Pro His Ile Lys Tyr Phe
610                 615                 620

Ile Asp Gly Lys Leu Ala Tyr Thr Glu His Thr Lys Val Pro Thr Met
625                 630                 635                 640

Ser Ala Arg Tyr Trp Ile Gly Val Trp Phe Pro Asn Asn Trp Ala Gly
                645                 650                 655

Asp Pro Asn Phe Glu Thr Asp Tyr Leu Glu Val Glu Tyr Phe Lys Tyr
            660                 665                 670

Glu Ser Phe Pro Asp His Pro Tyr Val Val Gly Pro Thr Gly Ala Ser
        675                 680                 685

Ser Pro Thr Ala Phe Tyr Pro Thr Ala Pro Ile Lys Lys Pro Val Ser
690                 695                 700

Asn Leu Leu Pro His Gly Asn Leu Asp Tyr Glu Thr Gly Tyr Met Leu
705                 710                 715                 720

Thr Gly Asp Ala Val Ile Ser Asn Gly Glu Leu Lys Thr Gly Leu Leu
                725                 730                 735

Gly Ser Ala Glu Ser Leu Ile Thr Gly Leu Asn Asp Ala Phe Glu Leu
            740                 745                 750

Thr Leu Lys Leu Lys Ala Lys Ala Ser Asn Asn Ala Thr Val Arg Ile
        755                 760                 765

Glu Tyr Leu Asp Lys Asp Leu Asn Val Ile Ser Gly Glu Asp Ile Ile
770                 775                 780

Val Ser Asn Leu Asn Ala Asn Thr Phe Thr Asn Phe Thr Ser Val Ile
785                 790                 795                 800

Asn Leu Val Glu Gly Thr Arg Ala Ile Asn Val Ile Phe Glu Gly Thr
                805                 810                 815

Asn Ile Thr Tyr Asp Asp Leu Phe Ile Asn Leu Thr His Lys Val Asn
            820                 825                 830
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<212> DNA
<213> EGT Optimized

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agcaccaccg tggcaccggg cgaacatacc ctggtggcga aagcgctgcc ggaaggcagc 180

aaccagcaga ttcgttttag cattcagggc attgtgagcg gcgtgagcat taccggcgat 240

aaactgaacg tgggcaacgc cgtggaagat ggcatgaaat ttaccgttgt ggcgaccagc 300

gtgtatgacc cgaccattcg tgccaccctg gaatttaccg tggttaacgt tggcgtggaa 360

gtggtggaaa ttcgtaccga agaagaactg cgcgcgattc ataccaacga aggcggcctg 420

agcctgagct atgtgctgat gaacgatatt gaactgaccg ccccgtggac cccgattggc 480

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gatgatggca cccatatcat tagcgccagc aactttgtta aagcggaaac ctatgccaat 2760

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<213> EGT Optimized

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attgatccga acatgtgggt gattggcaac agcaaatggg gcgtgggcaa cggcggcgtg 240

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ctgtatgatg gcccgctgcg tggcattggc aacacccatg gccgtcgtac cggcgccatg 360

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cgttttggca gcaccaccgc catgtggacc tattattatg ataacggcat gaaccacgaa 480

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gaagtgctgg tgagcagcca gagcagctat gtgccggatc atgccggcga atttaacatt 720

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<212> PRT
<213> Piscirickettsia salmonis

<400> 7

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            20                  25                  30

Asn Gln Leu Lys Ala Gln His Thr Gln Leu Gln Gln Gln Val Ala Asn
        35                  40                  45

Leu Gln Gly Gln Gly Gln Thr Thr Gly Ala Val His Val Gly Ala Val
    50                  55                  60

Gly Gly Glu Leu Ile Ser Glu Asn Asn Tyr Asp Gly Arg Gly Leu Asp
65                  70                  75                  80

Leu Leu Lys Ser Leu Ala Lys Ala Gly Ser Asn Ala Pro Leu Leu Thr
                85                  90                  95

Ile Gly Gly Thr Leu Glu Ala Asp Ala Gln Met Asn Arg Asn Gly Asn
            100                 105                 110

Val Gly Ser Gly Ser Thr Ser Gly Asp Pro Ser Gly Leu Asn Tyr Thr
        115                 120                 125

Asp Gly Thr Ser Ser Ser Ala Phe Tyr Leu Asp Thr Ala Arg Ile Asp
    130                 135                 140

Ile Leu Ala His Val Asn Asp Trp Val Asn Gly Glu Ile Ser Tyr Asp
145                 150                 155                 160

Leu Asn Gly Asp Ser Gly Leu His Thr Gly Ser Leu Leu Val Gly Asn
                165                 170                 175

Leu Asn Gln Leu Pro Val Tyr Gly Gln Ile Gly Lys Phe Tyr Pro Asp
            180                 185                 190

Ala Gly Leu Phe Glu Leu Ala Ser Asp Asp Val Tyr Ser Ser Ser Leu
```

```
        195                 200                 205

Val Lys Arg Tyr Phe Arg Pro Asp Ala Gln Asn Gly Ala Ser Val Gly
    210                 215                 220

Phe Tyr Lys Ala Gly Leu His Thr Ser Leu Thr Ala Phe Lys Thr Ser
225                 230                 235                 240

Ala Pro Gln Ala Asn Ala Ala Asn Tyr Asn Gln Ala Thr Ser Asp Trp
                245                 250                 255

Ser Ala Gln Ala Asp Tyr Thr Phe Asn Ala Gly Gln Val Asn Ala Thr
            260                 265                 270

Ile Gly Ala Gly Tyr Leu Ser Asn Met Val Asn Thr Asn Asp Ser Phe
        275                 280                 285

Thr Ala Thr Gly Ala Gly Thr Gly Thr Gln Lys Asp Arg Leu Pro Met
    290                 295                 300

Ala Asn Val Ser Ala Lys Ile Gly Phe Gly Pro Phe Glu Ala Leu Ala
305                 310                 315                 320

Thr Tyr Ala Gln Thr Leu Lys Gly Leu Ala Asn Thr Thr Gly Gly Thr
                325                 330                 335

Thr Lys Leu Lys Ala Phe Asp Leu Glu Gly Ala Tyr His Phe Gln Ala
            340                 345                 350

Val Lys Pro Met Thr Val Met Leu Gly Tyr Ser Arg Thr Tyr Gly Phe
        355                 360                 365

Asp Lys Val Gly Pro Val Asp Gln Phe Ile Asp Gly Asn Thr Ala Ile
    370                 375                 380

Thr Ile Asn Asn Lys Lys Asp Gln Trp Leu Leu Gly Val Asn Ser Glu
385                 390                 395                 400

Val Phe Lys Asn Thr Thr Val Gly Leu Glu Tyr Ala Arg Val Gly Gln
                405                 410                 415

Leu Asp Ser Thr Gly Thr Asp Thr Asn Arg Tyr Asn Val Leu Thr Ala
            420                 425                 430

Asp Met Thr Val Lys Phe
        435
```

<210> 8
<211> 416
<212> PRT
<213> Piscirickettsia salmonis

<400> 8

```
Ala Asp Asn Gly Lys Leu Gln Leu Gln Ile Asn Gln Leu Lys Ala Gln
1               5                  10                  15

His Thr Gln Leu Gln Gln Gln Val Ala Asn Leu Gln Gly Gln Gly Gln
            20                  25                  30

Thr Thr Gly Ala Val His Val Gly Ala Val Gly Gly Glu Leu Ile Ser
        35                  40                  45

Glu Asn Asn Tyr Asp Gly Arg Gly Leu Asp Leu Leu Lys Ser Leu Ala
    50                  55                  60

Lys Ala Gly Ser Asn Ala Pro Leu Leu Thr Ile Gly Gly Thr Leu Glu
65                  70                  75                  80

Ala Asp Ala Gln Met Asn Arg Asn Gly Asn Val Gly Ser Gly Ser Thr
                85                  90                  95

Ser Gly Asp Pro Ser Gly Leu Asn Tyr Thr Asp Gly Thr Ser Ser Ser
            100                 105                 110

Ala Phe Tyr Leu Asp Thr Ala Arg Ile Asp Ile Leu Ala His Val Asn
        115                 120                 125

Asp Trp Val Asn Gly Glu Ile Ser Tyr Asp Leu Asn Gly Asp Ser Gly
    130                 135                 140

Leu His Thr Gly Ser Leu Leu Val Gly Asn Leu Asn Gln Leu Pro Val
145                 150                 155                 160

Tyr Gly Gln Ile Gly Lys Phe Tyr Pro Asp Ala Gly Leu Phe Glu Leu
                165                 170                 175

Ala Ser Asp Asp Val Tyr Ser Ser Ser Leu Val Lys Arg Tyr Phe Arg
            180                 185                 190

Pro Asp Ala Gln Asn Gly Ala Ser Val Gly Phe Tyr Lys Ala Gly Leu
        195                 200                 205
```

```
His Thr Ser Leu Thr Ala Phe Lys Thr Ser Ala Pro Gln Ala Asn Ala
    210                 215                 220

Ala Asn Tyr Asn Gln Ala Thr Ser Asp Trp Ser Ala Gln Ala Asp Tyr
225                 230                 235                 240

Thr Phe Asn Ala Gly Gln Val Asn Ala Thr Ile Gly Ala Gly Tyr Leu
                245                 250                 255

Ser Asn Met Val Asn Thr Asn Asp Ser Phe Thr Ala Thr Gly Ala Gly
            260                 265                 270

Thr Gly Thr Gln Lys Asp Arg Leu Pro Met Ala Asn Val Ser Ala Lys
        275                 280                 285

Ile Gly Phe Gly Pro Phe Glu Ala Leu Ala Thr Tyr Ala Gln Thr Leu
    290                 295                 300

Lys Gly Leu Ala Asn Thr Thr Gly Gly Thr Thr Lys Leu Lys Ala Phe
305                 310                 315                 320

Asp Leu Glu Gly Ala Tyr His Phe Gln Ala Val Lys Pro Met Thr Val
                325                 330                 335

Met Leu Gly Tyr Ser Arg Thr Tyr Gly Phe Asp Lys Val Gly Pro Val
            340                 345                 350

Asp Gln Phe Ile Asp Gly Asn Thr Ala Ile Thr Ile Asn Asn Lys Lys
        355                 360                 365

Asp Gln Trp Leu Leu Gly Val Asn Ser Glu Val Phe Lys Asn Thr Thr
    370                 375                 380

Val Gly Leu Glu Tyr Ala Arg Val Gly Gln Leu Asp Ser Thr Gly Thr
385                 390                 395                 400

Asp Thr Asn Arg Tyr Asn Val Leu Thr Ala Asp Met Thr Val Lys Phe
                405                 410                 415
```

<210> 9
<211> 367
<212> PRT
<213> Piscirickettsia salmonis

<400> 9

```
Met Ala Thr Leu Ala Val Gln Arg Glu Val Tyr Met Ser Asp Pro Asp
1               5                   10                  15
```

```
Asp Ile Ala Val Ile Leu Tyr Thr Ser Gly Thr Thr Gly Gln Pro Lys
            20                  25                  30

Gly Ala Met Leu Ser His Arg Ala Leu Val Gln Asn Cys Ile Asp Leu
        35                  40                  45

Asn Leu Cys Trp Gly Phe Thr Asp Ser Asp Val Leu Leu His Thr Leu
    50                  55                  60

Pro Leu Phe His Val His Gly Leu Phe Phe Ala Leu His Ser Val Leu
65                  70                  75                  80

Tyr Ala Ser Ala Ser Met Ile Leu Gln Ala Lys Phe Asp Pro Met Glu
                85                  90                  95

Val Ile Ile Ser Leu Ile Gln Ala Thr Val Phe Met Gly Val Pro Thr
            100                 105                 110

Tyr Tyr Thr Arg Leu Leu Lys Glu Ala Glu Phe Thr Gly Ser Arg Ala
        115                 120                 125

Ala Gln Val Arg Leu Phe Ile Ser Gly Ser Ala Pro Leu His Glu Lys
    130                 135                 140

Thr Phe Gln Gly Phe Tyr Gln Arg Thr Gly Lys Thr Leu Val Glu Arg
145                 150                 155                 160

Tyr Gly Met Ser Glu Thr Gly Ile Asn Thr Ser Asn Pro Leu His Gly
                165                 170                 175

Glu Arg Lys Phe Gly Thr Val Gly Thr Ala Leu Glu His Val Thr Val
            180                 185                 190

Arg Val Val Asp Glu Val Ser Glu Lys Val Leu Met Pro Gly Gln Thr
        195                 200                 205

Gly Glu Val Gln Val Gln Gly Arg His Leu Phe Ser Gly Tyr Trp Gln
    210                 215                 220

Lys Glu Asp Gln Thr Asp Gly Ala Phe Thr Cys Asp Gln Phe Phe Lys
225                 230                 235                 240

Thr Gly Asp Leu Gly Tyr Leu Asp Glu Gln Gly Tyr Leu Thr Leu Val
                245                 250                 255
```

```
Gly Arg Val Lys Asp Met Ile Ile Ser Gly Gly Leu Asn Ile Tyr Pro
            260                 265                 270

Lys Glu Ile Glu Thr Ala Ile Asp Arg Val Thr Gly Val Asn Glu Ser
        275                 280                 285

Ala Val Val Gly Val Ala His Glu Asp Leu Gly Glu Gly Val Val Ala
    290                 295                 300

Val Val Val Leu Gln Asp Asn Ala Asn Met Leu Ala Glu His Ile Ile
305                 310                 315                 320

Ala Tyr Cys Lys Ala Ser Leu Ala Asp Phe Lys Cys Pro Lys Lys Val
                325                 330                 335

Val Phe Ile Asp Gln Leu Pro Arg Asn Thr Met Gly Lys Val Gln Lys
            340                 345                 350

Asn Gln Leu Arg Gln Gln Tyr Gln Ala Ile Phe Ala Asp Ala His
        355                 360                 365
```

<210> 10
<211> 39
<212> PRT
<213> Piscirickettsia salmonis

<400> 10

```
Met Cys Lys Ile Pro Thr Glu Val Ala Thr Leu Thr Ala Glu Leu Asn
1               5                   10                  15

Lys Arg Leu Arg Leu Asn Met Phe Ala Cys Leu Tyr Ile Asp Phe Ile
            20                  25                  30

Pro Pro Cys Ile Asn Arg Tyr
        35
```

<210> 11
<211> 127
<212> PRT
<213> Piscirickettsia salmonis

<400> 11

```
Met Lys Asn Leu Ile Tyr Ala Gln Arg Leu Leu Tyr Phe Ala Val Leu
1               5                   10                  15

Ile Ala Val Ile Val Thr Phe Val Gln Pro Phe Leu Met Pro Ile Lys
            20                  25                  30
```

```
Leu Ala Asp Val Pro Leu Met Pro Leu Val Val Ala Ser Ile Tyr Ser
        35                  40                  45

Leu Ile Phe Ala Ala Ala Leu Ala Leu Ala Ala Tyr Lys Leu Pro Ser
    50                  55                  60

Lys Ala Gly Trp Pro Arg Phe Leu Leu Val Ile Leu Phe Ile Gly Asp
65                  70                  75                  80

Ala Met Pro Ala Val Lys Asn Trp Leu Val Leu Trp His Thr Thr Glu
                85                  90                  95

Leu Phe Ala Ile Ile Tyr Leu Met Lys Leu Met Leu Met Leu Ala Ala
            100                 105                 110

Ile Leu Leu Ser Leu Ser Lys Leu Ala Arg Asp Phe Tyr Lys Cys
        115                 120                 125
```

<210> 12
<211> 74
<212> PRT
<213> Piscirickettsia salmonis

<400> 12

```
Met Asp Asn Ala Ser Phe His Lys Ser Lys Gly Val Lys Glu Ala Ile
1               5                   10                  15

Glu Asp Ala Gly Cys His Leu Leu Phe Leu Pro Pro Tyr Ser Pro Asp
            20                  25                  30

Leu Asn Pro Ile Glu His Val Trp Ser Pro Leu Lys Asn Arg Val Arg
        35                  40                  45

Met Lys Leu Asp Gln Asp Glu Ile Asn Leu Glu Thr Ala Leu Ser Gln
    50                  55                  60

Val Met Lys Ser Met Ser Glu Thr Ile Arg
65                  70
```

<210> 13
<211> 81
<212> PRT
<213> Piscirickettsia salmonis

<400> 13

```
Met Pro Ser Pro Tyr Ser Tyr Asp Leu Arg Ile Arg Ala Leu Lys Met
```

1 5 10 15

Ile Asp Glu Gly Ile Pro Ile Thr Gln Ile Ser Lys Leu Leu Lys Ile
20 25 30

Ser Arg Asp Thr Leu His Arg Trp Lys Asn Arg Arg Asp His Thr Gly
35 40 45

Asp Val Lys Ala Arg Phe Gly Tyr Gln Thr Gly Tyr Asn His Lys Ile
50 55 60

Ser Asp Met Lys Glu Phe Gln Lys Phe Ile Asp Arg Glu Phe Pro Gly
65 70 75 80

Ser

<210> 14
<211> 319
<212> PRT
<213> Piscirickettsia salmonis

<400> 14

Leu Lys Ser Gln Lys Pro Pro Gly Asn Leu Thr Tyr Asn Leu Ser Ala
1 5 10 15

Pro Trp Ser Gln Leu Met Ala Leu Met Ser Pro Arg Lys Ser Leu Ala
20 25 30

Arg Leu Ser Val Phe Leu Phe Gln Ser Gly Asp Phe Val Glu Lys Gly
35 40 45

Arg Pro Leu Val Gln Leu Asp Asp Arg Thr Glu Gln Ala Asn Leu Leu
50 55 60

Gln Tyr Lys Ala Lys Leu Lys Leu Asp Gln Leu Thr Tyr Asp Arg Asp
65 70 75 80

Arg Ser Leu Leu Lys Lys Asn Ala Ile Ser Arg Gln Asp Val Asp Thr
85 90 95

Ala Leu Thr Ser Leu Glu Gln Thr Lys Ala Gln Met Leu Ala Thr Glu
100 105 110

Val Ser Ile Ser Gln Lys Leu Ile Arg Ala Pro Phe Ser Gly Lys Ile
115 120 125

```
Gly Ile Arg Asn Val Asn Leu Gly Gln Tyr Ile Ser Pro Gly Thr Asn
    130                 135                 140

Ile Val Ser Leu Gln Ser Ile Asn Pro Leu His Val Asn Phe Ser Leu
145                 150                 155                 160

Pro Gln Glu Asp Met Asn Lys Ile Lys Leu Gly Gln Lys Ile Ser Ala
                165                 170                 175

His Val Asp Thr Phe Ala Gly Arg Glu Phe Thr Gly Thr Ile Thr Ala
            180                 185                 190

Met Asn Ser Glu Val Asp Ser Asn Thr Arg Thr Ile Glu Ile Gln Ala
        195                 200                 205

Ser Leu Pro Asn Pro Lys His Glu Leu Tyr Pro Gly Met Phe Thr Thr
    210                 215                 220

Val Gln Val Tyr Leu Pro Val Leu Pro Lys Val Leu Thr Leu Pro His
225                 230                 235                 240

Thr Ala Val Thr Tyr Thr Leu Tyr Gly Asn Ser Val Tyr Leu Ile Gln
                245                 250                 255

Leu Asn Gly Lys Lys Asn Gln Gln Gly Glu Pro Thr Gly Thr Val Thr
            260                 265                 270

Arg Ile Ser Ile Gln Thr Gly Asp Gln Arg Ser Asn Thr Val Val Ile
        275                 280                 285

Asn Lys Gly Leu Lys Ala Gly Asp Leu Ile Val Asp Gly Gly Gln Leu
    290                 295                 300

Lys Leu Glu Asn Gly Ala Ala Ile Ala Leu Lys Asn Thr Thr Gln
305                 310                 315
```

<210> 15
<211> 572
<212> PRT
<213> Piscirickettsia salmonis

<400> 15

```
Met Val Leu Ala Ile Gly Leu Val Val Asp Asp Ala Ile Ile Val Val
1               5                   10                  15

Glu Asn Val His Arg His Ile Glu Glu Gly Lys Gln Pro Phe Asp Ala
```

```
                20                  25                  30

Ala Leu Ile Gly Ala Arg Glu Ile Ala Ser Pro Val Ile Ala Met Thr
        35                  40                  45

Ile Thr Leu Ala Ala Val Tyr Ala Pro Ile Ala Phe Val Gly Gly Ile
    50                  55                  60

Thr Gly Ala Leu Phe Lys Glu Phe Ala Leu Thr Leu Ala Ala Ala Val
65                  70                  75                  80

Ile Val Ser Gly Val Ile Ala Leu Thr Leu Ser Pro Met Met Cys Ser
                85                  90                  95

Lys Leu Leu Val Ala Asp Asn Ala Asn Gly Gly Leu Ala His Trp Leu
            100                 105                 110

Asp Arg Gln Phe Leu Arg Leu Gln Gln Arg Tyr Glu Arg Ile Leu His
        115                 120                 125

His Thr Leu Glu His Arg Pro Val Val Leu Thr Phe Gly Leu Ile Ile
    130                 135                 140

Leu Val Gly Ile Phe Gly Met Leu Lys Met Thr Gln Lys Gln Leu Ala
145                 150                 155                 160

Pro His Glu Asp Gln Gly Phe Leu Ile Thr Phe Ala Ser Ala Pro Lys
                165                 170                 175

Tyr Ala Asn Ile Asn Tyr Val Glu Lys Tyr Ser Glu Glu Phe Ala Lys
            180                 185                 190

Ile Tyr Lys Ser Phe Pro Ala Ile Ala Asp Tyr Phe Ile Ile Asn Thr
        195                 200                 205

Thr Gly Ala Gly Thr Phe Pro Ser Gln Val Thr Ser Gly Ala Val Leu
    210                 215                 220

Lys Pro Trp Arg Asp Arg Ser Met Thr Thr Met Gln Leu Gln Pro Leu
225                 230                 235                 240

Leu Gln His Lys Leu Asn Gln Ile Thr Gly Leu Gln Ala Gln Ala Ile
                245                 250                 255

Gln Met Pro Ala Leu Pro Gly Pro Asp Gly Met Pro Ile Gln Phe Val
            260                 265                 270
```

```
Leu Thr Ser Thr Ala Asp Tyr Ser Val Leu Asn Asn Val Met Thr Lys
        275                 280                 285

Phe Lys Ala Ala Ala Asp Lys Ser Gly Leu Phe Leu Phe Ser Ser Ser
    290                 295                 300

Asp Leu Lys Phe Asn Lys Pro Lys Leu Asn Ile Ala Ile Asp Arg Ala
305                 310                 315                 320

Lys Ala Ala Gln Met Gly Ile Thr Met Gln Gln Ile Gly Ser Thr Leu
                325                 330                 335

Ser Thr Leu Leu Ser Gly Gly Lys Val Asn Tyr Phe Ser Leu Asp Gly
            340                 345                 350

Arg Ser Tyr Lys Val Ile Pro Gln Leu Ala Asp Asn Glu Arg Leu Thr
        355                 360                 365

Pro Gln Gln Leu Asn Asn Asn Tyr Ile Lys Thr Ala Ala Gly Ala Leu
    370                 375                 380

Ile Pro Leu Ser Thr Leu Ile Thr Leu Ser Thr Ser Ile Glu Pro Gly
385                 390                 395                 400

Thr Leu Asn Gln Phe Gln Gln Leu Asn Ser Ala Thr Leu Ser Ala Val
                405                 410                 415

Ala Met Pro Gly His Thr Asp Thr Glu Ala Leu Asn Phe Leu Lys Ala
            420                 425                 430

Gln Ala Thr Lys Leu Met Pro Lys Gly Met Ser Tyr Asn Phe Ser Gly
        435                 440                 445

Gln Ser Arg Thr Leu Val Gln Glu Gly Asn Ala Leu Ile Tyr Thr Phe
    450                 455                 460

Phe Phe Ala Leu Ile Met Ile Phe Leu Val Leu Ala Ala Gln Phe Glu
465                 470                 475                 480

Ser Phe Arg Asp Pro Phe Ile Ile Met Phe Thr Val Pro Met Ala Ile
                485                 490                 495

Phe Gly Ala Ala Ile Pro Met Ala Phe Gly Trp Thr Ser Leu Asn Ile
            500                 505                 510
```

```
Tyr Thr Glu Ile Gly Leu Val Thr Leu Ile Gly Leu Ile Thr Lys His
        515                 520                 525

Gly Ile Leu Met Val Gln Phe Ala Asn Asp Leu Gln Glu Gln Glu Gly
    530                 535                 540

Arg Asp Ile Arg Ser Ala Ile Glu His Ala Ala Gly Met Arg Leu Arg
545                 550                 555                 560

Pro Ile Leu Met Thr Thr Ala Ala Met Val Val Gly
                565                 570
```

<210> 16
<211> 2092
<212> DNA
<213> Piscirickettsia salmonis

<400> 16

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ccaagaacta tcaaaaacta tataggcaaa gtataaagtc tgaagcttaa cctttgctta     60
aatgtacatc aggcttaagg tgatttctgt tgagtatttt cagagtctta agctcaattt    120
aatctttctt aaggttgaaa acaggctaaa atcaacattt tgataaaatt attaattttt    180
ttttattgtt ctttttttaat cggtttttat cctaatttga tagatagtta tcgaaattca    240
ataagttttg tttttaattg aatttttttt acgagtttgg gttttacaaa gtgaatttac    300
ctggttatag tagccccagt tgcttaatag cacttaaatg tgtatccaga taaaaacaag    360
ttagggtaaa aagaatgaaa gtaaaaatga ttgttgcagc tgtagctgtt gcaggtttaa    420
cagcgactgc cgcaaatgcc gctgataatg gtaagcttca attacaaatc aaccaattga    480
aggcgcaaca cactcaactt caacagcaag ttgctaatct gcaaggtcaa ggccaaacta    540
ctggtgccgt tcacgttggc gctgttggtg gtgaactaat ctctgaaaat aactacgatg    600
gtcgtggctt agatcttctt aaatcattag cgaaagcagg cagcaatgca ccgttattaa    660
ctattggtgg tacgttagaa gctgatgcgc aaatgaaccg taacggtaat gttggatctg    720
gttctacttc tggtgaccct tctggcctta actatactga tggaactagc agttctgcat    780
tctatttaga tactgcacgt attgatatct tagcgcatgt gaatgactgg gttaacggtg    840
aaatctcgta tgacttaaat ggtgatagtg gtcttcacac tggtagcctt ttagtgggta    900
acctcaatca attaccagtt tatggtcaaa tcggtaaatt ctacccagat gcaggtttgt    960
ttgaattagc tagtgatgat gtttattctt ctagcttagt caagcgttat ttccgtccag   1020
atgcgcaaaa tggtgcatct gtaggcttct ataaagcagg cttacatact tctttaactg   1080
catttaaaac gtctgctcca caagctaatg ctgctaacta taaccaagca actagtgatt   1140
```

```
ggtctgcaca agcggattac acttttaatg caggtcaagt caatgccact ataggtgcag   1200
gttacttatc taatatggtg aataccaatg acagcttcac tgcaacaggt gcaggaactg   1260
gtacacaaaa agatcggcta ccgatggcta atgtaagcgc taagattggc tttggtccat   1320
ttgaagccct tgctacttat gctcaaacat taaaaggttt ggcgaatact acaggtggta   1380
caacgaagtt gaaagccttt gatttagaag gtgcttacca cttccaagct gtgaagccga   1440
tgactgtgat gttaggttat agccgtacat atggctttga taaggttgga cctgttgatc   1500
agtttattga tggtaatact gcgattacta tcaataacaa aaaagaccaa tggttattgg   1560
gtgtaaactc tgaagtattt aagaacacaa cggttggtct tgagtatgcg cgtgtaggtc   1620
agcttgatag cacaggtact gacactaacc gctacaacgt attgactgcg gatatgactg   1680
ttaagttcta atttaagagc tttaaagttt tcaaaaaggc gctgcggcgc ctttttttat   1740
gggcgttaat tattggtaat gtaggctagt atttaaattt gtgagtgatg agagatgaaa   1800
aatttaatct atgcacagcg tttgctttat tttgccgtat tgattgcggt gattgtcacc   1860
tttgttcagc catttctaat gccgattaag cttgctgatg tgcctttaat gccgctcgtg   1920
gtcgcttcga tttattcctt gatttttgct gcagctttag cattagctgc atataaatta   1980
ccgagcaaag ctggttggcc gcggtttttg ttggtgattt tatttattgg ggatgcgatg   2040
cctgcggtaa aaaactggct agtgctttgg catacgacgg agctttttgc ga            2092
```

Claims

1. A $^{Ps}p_1$90 protein that has an amino acid sequence comprising at least 75% identity with the amino acid sequence of SEQ ID NO; 2 and that is capable of eliciting antibodies that are protecting fish from *Piscirickettsia salmonis* infection, wherein said protein is in a form selected from the group consisting of isolated, recombinant, or both isolated and recombinant.

2. The protein of claim 1, **characterised in that** the amino acid sequence is selected from the group consisting of SEQ ID NO; 2 and SEQ ID NO: 2 comprising one or more conservative amino acid substitutions.

3. An antigenic fragment of the $^{Ps}p_1$90 protein **characterised in that** said antigenic fragment has the amino acid sequence of SEQ ID NO: 2.

4. A chimeric polypeptide **characterised in that** said chimeric protein comprises the antigenic fragment of claim 3.

5. An antibody that binds specifically to the $^{Ps}p_1$90 protein of claim 1, or to an antigenic fragment of said protein.

6. A nucleic acid **characterised in that** said nucleic acid encodes the $^{Ps}p_1$90 protein of claim 1, **characterised in that** said nucleic acid is in a form selected from the group consisting of isolated, recombinant, or both isolated and recombinant.

7. The nucleic acid of claim 6, **characterised in that** it comprises a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, a nucleotide sequence of a DNA molecule that hybridizes under stringent conditions with the nucleic acid having the nucleotide sequence of SEQ ID NO: 1, and the nucleotide

sequence of SEQ ID NO; 5.

8. An expression vector comprising the nucleic acid of claim 7.

9. The expression vector of claim 8 that is the EGT1 plasmid having the BCCM accession No. LMBP 5690.

10. A host cell that comprises the expression vector of claim 8.

11. A method for producing a recombinant $^{Ps}p_1$90 protein comprising culturing the host cell of claim 10 in a culture medium.

12. The method of claim 11 that further comprises isolating the $^{Ps}p_1$90 protein.

13. The method of claim 12 wherein the host cell is an E. coli cell.

14. An antigenically effective amount of the $^{Ps}p_1$90 protein of claim 1 or a mixture of an antigenically effective amount of said $^{Ps}p_1$90 protein and an antigenically effective amount of isolated and/or recombinant $^{Ps}p_2$90 protein that has an amino acid sequence having at least 75% identity with the amino acid sequence of SEQ ID NO: 4, and a pharmaceutically acceptable carrier, for use as a vaccine.

15. The antigenically effective amount of isolated $^{Ps}p_1$90 protein according to claim 14 for use as a vaccine according to claim 14 **characterised in that** said vaccine further comprises a ^{Ps}45 protein or antigenic fragment thereof.

16. The antigenically effective amount of isolated $^{Ps}p_1$90 protein according to claim 14 for use as a vaccine according to claim 14, **characterised in that** said vaccine further comprises an antigen obtained from an Infectious Pancreatic Necrosis (IPN) virus.

17. The antigenically effective amount of isolated $^{Ps}p_1$90 protein according to claim 16 for use as a vaccine according to claim 16, **characterised in that** the antigen obtained from the IPN virus is selected from the group consisting of the VP2 var protein and the VP3 protein.

18. The antigenically effective amount of isolated $^{Ps}p_1$90 protein according to claim 17 for use as a vaccine according to claim 17, **characterised in that** said vaccine further comprises both the VP2 var protein and the VP3 protein from Infectious Pancreatic Necrosis (IPN) virus.

19. The antigenically effective amount of isolated $^{Ps}p_1$90 protein according to claim 14 for use as a vaccine according to claim 14 **characterised in that** said vaccine further comprises an antigen obtained from *Aeromonas salmonicida.*

20. A $^{Ps}p_1$90 protein according to claim 1 or 2, or an antigenic fragment thereof according to claim 3, for use in the treatment of salmonid rickettsial septicaemia in fish.

21. The $^{Ps}p_1$90 protein according to claim 1 or 2, or an antigenic fragment thereof according to claim 3, for use in the treatment according to claim 20, wherein the fish is a teleost.

22. The $^{Ps}p_1$90 protein according to claim 1 or 2, or an antigenic fragment thereof according to claim 3, for use in the treatment according to claim 21, wherein the teleost is a salmonid.

23. The $^{Ps}p_1$90 protein according to claim 1 or 2, or an antigenic fragment thereof according to claim 3, for use in the treatment according to claim 22, wherein the salmonid is selected from the group consisting of *Salmo salar, Oncorhynchus kisutch* and *Oncorhynchus mykiss.*

Patentansprüche

1. $^{Ps}p_1$90-Protein, das eine Aminosäuresequenz mit wenigstens 75% Identität mit der Aminosäuresequenz von SEQ ID NO: 2 aufweist und das Antikörper hervorrufen kann, die Fische vor einer Infektion mit *Piscirickettsia salmonis* schützen, wobei das Protein in einer aus der aus isoliert, rekombinant oder sowohl isoliert als auch rekombinant bestehenden Gruppe ausgewählten Form vorliegt.

2. Protein nach Anspruch 1, **dadurch gekennzeichnet, dass** die Aminosäuresequenz aus der aus SEQ ID NO: 2 und SEQ ID NO: 2 mit einer oder mehreren konservativen Aminosäuresubstitutionen bestehenden Gruppe ausgewählt ist.

3. Antigenes Fragment des $^{Ps}p_1$90-Proteins, **dadurch gekennzeichnet, dass** das antigene Fragment die Aminosäuresequenz von SEQ ID NO: 2 aufweist.

4. Chimäres Polypeptid, **dadurch gekennzeichnet, dass** das chimäre Protein das antigene Fragment nach Anspruch 3 umfasst.

5. Antikörper, der spezifisch an das $^{Ps}p_1$90-Protein nach Anspruch 1 oder an ein antigenes Fragment des Proteins bindet.

6. Nukleinsäure, **dadurch gekennzeichnet, dass** die Nukleinsäure das $^{Ps}p_1$90-Protein nach Anspruch 1 codiert, **dadurch gekennzeichnet, dass** die Nukleinsäure in einer aus der aus isoliert, rekombinant oder sowohl isoliert als auch rekombinant bestehenden Gruppe ausgewählten Form vorliegt.

7. Nukleinsäure nach Anspruch 6, **dadurch gekennzeichnet, dass** sie eine aus der aus der Nukleotidsequenz von SEQ ID NO: 1, einer Nukleotidsequenz eines DNA-Moleküls, das unter stringenten Bedingungen mit der die Nukleotidsequenz von SEQ ID NO: 1 aufweisenden Nukleinsäure hybridisiert, und der Nukleotidsequenz von SEQ ID NO: 5 bestehenden Gruppe ausgewählte Nukleotidsequenz umfasst.

8. Expressionsvektor, umfassend die Nukleinsäure nach Anspruch 7.

9. Expressionsvektor nach Anspruch 8, bei dem es sich um das EGT1-Plasmid mit der BCCM-Zugangsnummer LMBP 5690 handelt.

10. Wirtszelle, die den Expressionsvektor nach Anspruch 8 umfasst.

11. Verfahren zur Herstellung eines rekombinanten $^{Ps}p_1$90-Proteins, wobei man die Wirtszelle nach Anspruch 10 in einem Kulturmedium kultiviert.

12. Verfahren nach Anspruch 11, bei dem man ferner das $^{Ps}p_1$90-Protein isoliert.

13. Verfahren nach Anspruch 12, wobei es sich bei der Wirtszelle um eine E. coli-Zelle handelt.

14. Antigen wirksame Menge des $^{Ps}p_1$90-Proteins nach Anspruch 1 oder Gemisch einer antigen wirksamen Menge des $^{Ps}p_1$90-Proteins und einer antigen wirksamen Menge isolierten und/oder rekombinanten $^{Ps}p_2$90-Proteins, das eine Aminosäuresequenz mit wenigstens 75% Identität mit der Aminosäuresequenz von SEQ ID NO: 4 aufweist, sowie ein pharmazeutisch unbedenklicher Trägerstoff zur Verwendung als Impfstoff.

15. Antigen wirksame Menge isolierten $^{Ps}p_1$90-Proteins gemäß Anspruch 14 zur Verwendung als Impfstoff gemäß Anspruch 14, **dadurch gekennzeichnet, dass** der Impfstoff ferner ein ^{PS}45-Protein oder ein antigenes Fragment davon umfasst.

16. Antigen wirksame Menge isolierten $^{Ps}p_1$90-Proteins gemäß Anspruch 14 zur Verwendung als Impfstoff gemäß Anspruch 14, **dadurch gekennzeichnet, dass** der Impfstoff ferner ein aus einem IPN(Infectious Pancreatic Necrosis)-Virus gewonnenes Antigen umfasst.

17. Antigen wirksame Menge isolierten $^{Ps}p_1$90-Proteins gemäß Anspruch 16 zur Verwendung als Impfstoff gemäß Anspruch 16, **dadurch gekennzeichnet, dass** das aus dem IPN-Virus gewonnene Antigen aus der aus dem VP2-var-Protein und dem VP3-Protein bestehenden Gruppe ausgewählt ist.

18. Antigen wirksame Menge isolierten $^{Ps}p_1$90-Proteins gemäß Anspruch 17 zur Verwendung als Impfstoff gemäß Anspruch 17, **dadurch gekennzeichnet, dass** der Impfstoff ferner sowohl das VP2-var-Protein als auch das VP3-Protein aus IPN(Infectious Pancreatic Necrosis)-Virus umfasst.

19. Antigen wirksame Menge isolierten $^{Ps}p_1$90-Proteins gemäß Anspruch 14 zur Verwendung als Impfstoff gemäß

Anspruch 14, **dadurch gekennzeichnet, dass** der Impfstoff ferner ein aus *Aeromonas salmonicida* gewonnenes Antigen umfasst.

20. $^{Ps}p_1$90-Protein gemäß Anspruch 1 oder 2 oder antigenes Fragment davon gemäß Anspruch 3 zur Verwendung bei der Behandlung von Salmoniden-Rickettsien-Septikämie bei Fischen.

21. $^{Ps}p_1$90-Protein gemäß Anspruch 1 oder 2 oder antigenes Fragment davon gemäß Anspruch 3 zur Verwendung bei der Behandlung gemäß Anspruch 20, wobei es sich bei dem Fisch um einen Teleosteer handelt.

22. $^{Ps}p_1$90-Protein gemäß Anspruch 1 oder 2 oder antigenes Fragment davon gemäß Anspruch 3 zur Verwendung bei der Behandlung gemäß Anspruch 21, wobei es sich bei dem Teleosteer um einen Salmoniden handelt.

23. $^{Ps}p_1$90-Protein gemäß Anspruch 1 oder 2 oder antigenes Fragment davon gemäß Anspruch 3 zur Verwendung bei der Behandlung gemäß Anspruch 22, wobei der Salmonide aus der aus *Salmo salar, Oncorhynchus kisutch* und *Oncorhynchus mykiss* bestehenden Gruppe ausgewählt ist.

Revendications

1. Protéine $^{Ps}p_1$90 qui a une séquence d'acides aminés comprenant au moins 75 % d'identité avec la séquence d'acides aminés de SEQ ID NO: 2 et qui est capable d'induire des anticorps qui protègent les poissons contre une infection par *Piscirickettsia salmonis,* ladite protéine étant sous une forme choisie dans le groupe constitué d'isolée, recombinante, ou à la fois isolée et recombinante.

2. Protéine de la revendication 1, **caractérisée en ce que** la séquence d'acides aminés est choisie dans le groupe constitué de SEQ ID NO: 2 et SEQ ID NO: 2 comprenant une ou plusieurs substitutions d'acide aminé conservatrices.

3. Fragment antigénique de la protéine $^{Ps}p_1$90 **caractérisé en ce que** ledit fragment antigénique a la séquence d'acides aminés de SEQ ID NO: 2.

4. Polypeptide chimère **caractérisé en ce que** ladite protéine chimère comprend le fragment antigénique de la revendication 3.

5. Anticorps qui se lie spécifiquement à la protéine $^{Ps}p_1$90 de la revendication 1, ou à un fragment antigénique de ladite protéine.

6. Acide nucléique **caractérisé en ce que** ledit acide nucléique code pour la protéine $^{Ps}p_1$90 de la revendication 1, **caractérisé en ce que** ledit acide nucléique est sous une forme choisie dans le groupe constitué d'isolé, recombinant, ou à la fois isolé et recombinant.

7. Acide nucléique de la revendication 6, **caractérisé en ce qu'**il comprend une séquence nucléotidique choisie dans le groupe constitué de la séquence nucléotidique de SEQ ID NO: 1, une séquence nucléotidique d'une molécule d'ADN qui s'hybride dans des conditions stringentes avec l'acide nucléique ayant la séquence nucléotidique de SEQ ID NO: 1, et la séquence nucléotidique de SEQ ID NO: 5.

8. Vecteur d'expression comprenant l'acide nucléique de la revendication 7.

9. Vecteur d'expression de la revendication 8 qui est le plasmide EGT1 ayant le numéro d'ordre BCCM n° LMBP 5690.

10. Cellule hôte qui comprend le vecteur d'expression de la revendication 8.

11. Procédé pour produire une protéine $^{Ps}p_1$90 recombinante comprenant la culture de la cellule hôte de la revendication 10 dans un milieu de culture.

12. Procédé de la revendication 11 qui comprend en outre l'isolement de la protéine $^{Ps}p_1$90.

13. Procédé de la revendication 12 dans lequel la cellule hôte est une cellule de *E. coli.*

14. Quantité efficace sur le plan antigénique de la protéine $^{Ps}p_1$90 de la revendication 1 ou un mélange d'une quantité efficace sur le plan antigénique de ladite protéine $^{Ps}p_1$90 et d'une quantité efficace sur le plan antigénique de protéine $^{Ps}p_2$90 isolée et/ou recombinante qui a une séquence d'acides aminés ayant au moins 75 % d'identité avec la séquence d'acides aminés de SEQ ID NO: 4, et un véhicule pharmaceutiquement acceptable, pour utilisation en tant que vaccin.

15. Quantité efficace sur le plan antigénique de protéine $^{Ps}p_1$90 isolée selon la revendication 14 pour utilisation en tant que vaccin selon la revendication 14 **caractérisée en ce que** ledit vaccin comprend en outre une protéine ^{PS}45 ou un fragment antigénique de celle-ci.

16. Quantité efficace sur le plan antigénique de protéine $^{Ps}p_1$90 isolée selon la revendication 14 pour utilisation en tant que vaccin selon la revendication 14, **caractérisée en ce que** ledit vaccin comprend en outre un antigène obtenu à partir d'un virus de nécrose pancréatique infectieuse (IPN).

17. Quantité efficace sur le plan antigénique de protéine $^{Ps}p_1$90 isolée selon la revendication 16 pour utilisation en tant que vaccin selon la revendication 16, **caractérisée en ce que** l'antigène obtenu à partir du virus IPN est choisi dans le groupe constitué de la protéine VP2 var et la protéine VP3.

18. Quantité efficace sur le plan antigénique de protéine $^{Ps}p_1$90 isolée selon la revendication 17 pour utilisation en tant que vaccin selon la revendication 17, **caractérisée en ce que** ledit vaccin comprend en outre à la fois la protéine VP2 var et la protéine VP3 du virus de nécrose pancréatique infectieuse (IPN).

19. Quantité efficace sur le plan antigénique de protéine $^{Ps}p_1$90 isolée selon la revendication 14 pour utilisation en tant que vaccin selon la revendication 14 **caractérisée en ce que** ledit vaccin comprend en outre un antigène obtenu à partir *d'Aeromonas salmonicida.*

20. Protéine $^{Ps}p_1$90 selon la revendication 1 ou 2, ou fragment antigénique de celle-ci selon la revendication 3, pour utilisation dans le traitement de la septicémie rickettsienne des salmonidés chez les poissons.

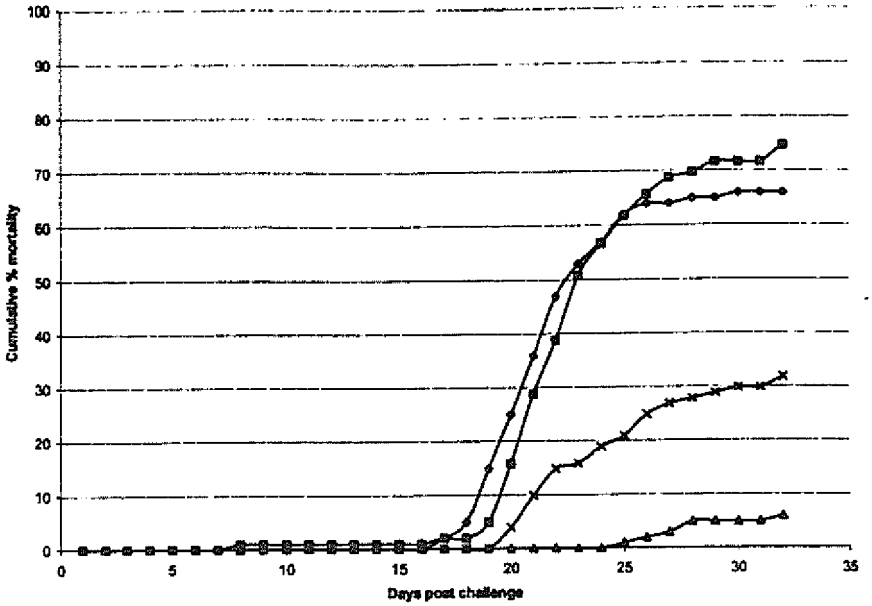
21. Protéine $^{Ps}p_1$90 selon la revendication 1 ou 2, ou fragment antigénique de celle-ci selon la revendication 3, pour utilisation dans le traitement selon la revendication 20, le poisson étant un téléostéen.

22. Protéine $^{Ps}p_1$90 selon la revendication 1 ou 2, ou fragment antigénique de celle-ci selon la revendication 3, pour utilisation dans le traitement selon la revendication 21, le téléostéen étant un salmonidé.

23. Protéine $^{Ps}p_1$90 selon la revendication 1 ou 2, ou fragment antigénique de celle-ci selon la revendication 3, pour utilisation dans le traitement selon la revendication 22, le salmonidé étant choisi dans le groupe constitué de *Salmo salar, Oncorhynchus kisutch* et *Oncorhynchus mykiss.*

FIGURE 1

Cumulative mortality



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