DNA-based vaccines against bacterial fish diseases: trials and prospective

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Abstract: Bacterial diseases of cultured fish considered the most impediments on aquaculture development causing high mortalities and huge economic losses. The aim of this review is to collect the dispersed literatures published about live attenuated, subunit and DNA vaccines against vibriosis, photobacteriosis, furunculosis, motile aeromonas septicaemia, pseudomonadiasis, yersiniosis, edwardsiellosis, enteric septicaemia of catfish, cold-water disease, columnaris disease, streptococcosis and lactococcosis. With advances in molecular biology, genetically modified vaccines have been increasingly employed against many of the fish pathogens. It is expected that some of them may be commercialized in the near future.
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1. Introduction

Prevention of fish diseases by inactivated vaccines have been documented for many bacterial diseasesadministered by immersion or intraperitoneal injection. While genetically modified vaccinesdisplay anadvantage of enabling more targetedsafer vaccines especially against intracellular pathogens [1,2]. TheDNA vaccine preparations stimulatestrong an antibody response and cellular immunity[3]. There are many trials to develop DNA vaccines against fish pathogens, while there are a limited number of DNA vaccine strategies that have been successful in giving significant protection. The safety of DNA vaccines has been questioned for some time. After a high level of protection against the rhabdovirusesviral hemorrhagic septicaemia virus and infectious hematopoieticvirus in salmonids, DNA vaccines seemed to be more promising[3,4].

This review will focus on the research efforts to develop effective DNA vaccines againstbacterial fish diseases based on virulence factors, which there is currently no licensed DNA vaccines available. In addition, it will focus on improvement of vaccine efficacy using specific adjuvants, vectors and delivery routes.

Trials of using live attenuated and DNA-based vaccines against bacterial diseases

1. Vibriosis

Vibrio species are g-ve bacteria of the family Vibrionaceae, the causative agent of vibriosis. Vibriosis is a deadly haemorrhagic septicaemic disease affectingvarious marine and fresh/brackish water fish, bivalves and crustaceanscausingsevere economic losses worldwide [5,6]. Within the genus Vibrio, the species causing the most economically serious diseases in aquaculture are; *V. anguillarum, V. harveyi*, *V. alginolyticus, V. vulnificus, V. parahaemolyticus, V. ordalii, V. Salmonicida*and *V.mimicus*.

a- Vibrio anguillarum

Multiple commercial vaccines have been developed to protectfish against outbreaks of vibriosisby formalin killed bacteria, heat-inactivated*V. anguillarum*cells and*V. Anguillarum* bacterin, for exampleMICROViB (Microtek International Inc.), ALPHAMARINE Vibrio (PHARMAQ AS), and AquaVac Vibrio and Norvax Vibriose Marine (Schering-Plough Aquaculture). All these vaccines consistof inactivated strains of both *V. anguillarum* serotypes O1 and O2 and show good protection against*V. anguillarum* infections in several fish species[5].

*V. anguillarum*have some virulence-relatedfactors, including genes affecting chemotaxis andmotility, flagellin D[7], adhesins (pili, fimbriae, outer membrane proteins, LPS, extracellular polysaccharides), Invasion of host tissues (Protease, Haemolysin), iron uptake system (Siderophore-dependent, Siderophore-independent), and quorum sensing which could be the basis for development of DNA vaccines against *V. anguillarum*[5]. DNA vaccines were constructed using the major outer membrane proteins OMP38, a divalent DNA vaccine based on Sia10 of *S. iniae*and OmpU of *V. anguillarum*, zinc metalloprotease EmpAanda rigorous iron-regulatedpromoter P_{viuA} to control the expression of phage P22 lysiscassette 13-19-15 (Table 1).

b- Vibrio harveyi

V. harveyi, is the causativeagent of luminous vibriosis, a serious disease of shrimp responsible for heavy economic losses worldwide. Also, can affect

lobster, abalone, finfish, and oyster especiallyin South America and Asia. Antibiotics can treat *V. harveyi* efficiently. Trials for test candidate vaccines, in the forms ofbacterin and subunit vaccines, have been reported but until now no licensed vaccinesagainst *V. harveyi*[14]. Several virulence factors have shown to participate in pathogenicity of *V. harveyi* such as outer membraneprotein OmpU and OmpK, cytotoxic proteases, hemolysins, lipases and phospholipases, type III secretion system, siderophore production, and Quorum-sensingwhich could be the basis for development of DNA vaccines against *V. Harveyi* (Table 2).

Table 1: DNA and live attenuated vaccines trials against *V. anguillarum*

Vaccine	Adjuvant	Species	Route	RPS%	Ref.
Liveattenuated iron-regulatedpromoter P_{viuA}	-	Zebrafish	ip	89.3	[8]
A divalent DNA Sia10 and OmpU *S. iniae* and *V. anguillarum*	-	Japanese flounder	im	78-81	[9]
*V. anguillarum*emp A and GAPDH from *A. hydrophila*	FCA	Turbot	ip	84	[10]
Mutated zinc-metalloprotease geneEmpA (m-EmpA7)	-	Japanese flounder	im	57.5-85.7	[11]
OMP38 DNA vaccine	-	Asian Seabass	im	55.6	[12]
Recombinant Aha1 adhesin from *A. hydrophila*	FCA	Blue gourami	ip	44.4	[13]

ip: intraperitoneal im: intramuscular

Table 2: DNA and live attenuated vaccines trials against *V. harveyi*

vaccine	adjuvant	Species	Route	RPS	Ref.
Dihydrolipoamide dehydrogenase (DLD)	-	Orange-spotted Grouper	ip	86	[15]
Recombinant LamB against different Vibrio spp.	-	Zebrafish	ip	60	[16]
Subunit OmpU encoded a35 kDa protein	B. subtilis cells	Turbot	im	100	[17]
Bivalent DNA vaccine of DegQ and Vhp1	-	Japaneseflounder	im	84.6	[14]
Recombinant cytotoxic protease Vhp1	Bacillus sp. B187,	Japanese flounder	ip	70	[18]
Recombinant OmpK of *V. harveyi*	FIA	Orange-spotted groupers	ip	66.7-100	[19]
Live attenuated *P. fluorescens*fur mutant and pJAQ plasmid of *V. harveyi* (TFM/pJAQ)	-	Japanese flounder	ip	82.2	[20]
OmpK (28 kDa)	FIA	Orange-spotted groupers	ip	100	[21]

c- Vibrio alginolyticus

*V.alginolyticus*one of the family Vibrionaceae with a broad host rangeof cultured marine animals includes shellfish, shrimp, and fish of various species and has brought a large damage in the economy. It is one of zoonotic importance isolated from clinical cases in humans. Althoughseveral trials have been made, there is no specific vaccine availableagainst *V. alginolyticus*. In addition, commercial vaccine products of other *Vibrio* spp. are not effective in preventing *V. alginolyticus*infections[22,23]. Many virulence factors been identified in *V. alginolyticus*as candidates for vaccination preparations such as outer membrane proteins, flagellin, hemolysin, and Type III secretion system (T3SS) (Table 3).

d- Vibrio parahaemolyticus

V. parahaemolyticus is a halophilc bacterium inhabits marine and estuarine environments worldwide. *V. parahaemolyticus*causes diseases inmarine fishes, shrimps and other crustaceans worldwide responsible for economic losses of the commercial aquaculture. In addition, *V. parahaemolyticus* considers as one offoodborne pathogens that causes human acute gastroenteritis associated with the consumption ofraw or under cooked seafood. *V. parahaemolyticus*possess wide variety of virulence factors such as thermostable direct hemolysin, thermostable direct hemolysin related hemolysin, adhesins, lethaltoxin, extracellular proteases, urease and type III secretion systems[30] (Table 4).

Table 3: DNA and live attenuated vaccines trials against *V. alginolyticus*

Vaccine	Adjuvant	Species	Route	RPS%	Ref.
Dihydrolipoamide dehydrogenase (DLD)	-	Orange-spotted Grouper	ip	90	[15]
Subunit vaccine of LPS	-	Silver sea bream	ip	100	[22]
Recombinant LamB against different Vibrio spp.	-	Zebrafish	ip	77.8	[16]
Outermembrane protein-OmpU	FIA	Crimson snapper	im	93.33	[23]
Recombinant VscO	Formalin	Grouper	ip	80	[24]
hfq deletion mutantstress resistance	-	Zebrafish	im	77.3	[25]
hfq deletion mutantstress resistance	-	Grouper	im imr	45–78.3 66.7	[25]
Recombinant flaA gene	-	Red snapper		88	[26]
Recombinant FlaC	-	Red snapper	ip	84	[27]
Recombinant thermolabile hemolysin (TLH)	-	Zebrafish	ip	-	[28]
Recombinant OmpK of *V. harveyi*	FIA	Orange-spotted groupers	ip	40-65.4	[19]
Recombinant outer membrane proteins, VA1061, OmpU, VPA1435 and VPA0860	FCA	Carp	ip	62.5 - 95	[29]

imr: immersion

Table 4: DNA and live attenuated vaccines trials against *V. parahaemolyticus*

Antigen	Adjuvant	Species	Route	RPS%	Ref.
Recombinant fusion protein transduction domain-outer membrane protein (PTD-ompK)	-	Marbled eel	ip imr	*	[31]
Recombinant LamB against different Vibrio spp.	-	Zebrafish	ip	62.5	[16]
DNA vaccine (ompK)	Chitosan particles encapsulated	Black seabream	oral	72.3	[32]
Recombinant DNA vaccine of mutated serine protease (Ser318ePro)	-	Turbotjuveniles	im	96.11	[30]
Recombinant OmpK of *V. harveyi*	FIA	Orange-spotted groupers	ip	50	[19]
Recombinant outer membranes OmpW, OmpV, OmpK, OmpU	-	Large yellow croaker	ip	80-90	[33]
Dihydrolipoamide dehydrogenase (DLD)	-	Orange-spotted Grouper	ip	80	[15]

(*) marbled eels immunized with PTD-ompK and challenged with deadly dose of *V. parahaemolyticus*survived significantly longer than those immunized with ompK alone did.

e- Vibrio vulnificus

*V. vulnificus*biotype 2 is a primary pathogen for eels aquaculture. While, *V. vulnificus* biotype 1 is an opportunistic humanpathogen causing disease after handling or ingestion of raw shellfish. Vulnivaccine is a bacterin from serovar E against *V. vulnificus* used in Spain to protect eel but gave short protection period for approximately 1 month[34]. A bivalent vaccine against serotype E and A designed by [35] against the two pathogenic serovars in eel vaccinated by oral, anal intubation, intraperitoneal and prolonged immersion. The results indicated that the new vaccine delivered by oral and anal intubation is much better than intraperitoneal injection by 80% higher in protection.

One trial to develop a novel recombinant bivalent outer membrane protein (OMP)of *V. vulnificus* and *A. hydrophila* vaccine was injectedintraperitoneally in American eel (*Anguilla rostrata*). The relative percent survival (RPS) of the fish after challenged with *A.hydrophila* and *V.vulnificus* were 50% and 50% respectively [36]. As the *V.vulnificus*is an important cause of fatal septicemia in human, a trial to develop a live attenuated vaccine with deletions in three major virulence factors: RTX cytotoxin gene, metalloprotease (vvpE) and hemolysin/cytolysin (vvhA). Intragastric immunizated mice showed systemic and mucosal immunity and protected from challenged virulent *V.vulnificus*through various injection routes[37].

f- Vibriomimicus

*V.mimicus*is extracellular bacteria that inhabits diverse aquatic environments causing ascites disease. It is also isolated from human with gastroenteritis after ingestion of raw or undercooked fish products. *V. mimicus* is most similar to *V. cholerae*in having the same virulence factors, such as enterotoxins andhemolysins[38] (Table 5).

Table 5: DNA vaccine trials against *V.mimicus*

Antigen	Adjuvent	Species	Route	RPS%	Ref.
Recombinant tandemly arranged outermembrane protein U (OmpU) multi-epitope (6EPIS)	ISA763A	Grass carp	ip	85.71	[38]
Recombinant LamB against different Vibrio spp.	-	Zebrafish	ip	54.1	[16]

2. Photobacteriosis (Pasteurellosis)

Photobacteriosis (Pasteurellosis), is caused by *Photobacterium damselae* subsp. *piscicida* (formerly *Pasteurella piscicida*), whichcauses Severe mortalities occur usually when water temperatures are above 18-20ºC among marine fishes worldwide. This bacterium is a member of the family *Vibrionaceae*, and similar to *P. damselae* subsp. *damselae.* To date, severaltypes of commercial vaccines have been reported, including bacterin, LPS formulations and ECP-enriched bacterin preparation; with poor protection. Thelicensed ECP-enriched bacterin (DI vaccine) has been employedin several European countries with mixed results rangingfrom good in Spainin larvae of gilthead sea bream, to poor in Italy[34,39,40]. Major virulence factors in *P. damselae* subsp. *piscicida* are themetalloprotease, Siderophore, outer membrane, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), andlipoprotein [39,41] which could be the basis for vaccine development (Table 6).

Table 6: DNA and live attenuated vaccines trials against*Photobacterium damselae* subsp. *piscicida.*

Vaccine	Adjuvant	Species	Route	RPS%	Ref.
DNA vaccineencoding codon-optimized PPA1 (a major antigenic protein)	-	Japanese flounder	im	90.9	[42]
Recombinant Lipoprotein subunitvaccine	FCA	Sea bass	ip	50	[40]
Recombinant rHSP60, rENOLASE, and rGAPDH antigens,	FCA	Cobia	ip	25-52[a] 48.4-65.6[b] 1.6[c]	[43]
Formalin-killed bacterin withEscherichia coli LPS	-	Sea bream	imr	72.2[d] 70.8[e]	[44]
Live attenuated aroA mutant	-	Hybrid striped bass	ip	85.5	[45]

([a]) MonovalentrHSP60, or rENOLASE, or rGAPDH, ([b]) divalent vaccine, ([c]) trivalent vaccine
(d) Sea bream larvae from immunized parents, (e) Larvae from non-immunized parents

3. Furunculosis

Typical furunculosis is caused by*Aeromonas salmonicida* subsp. *salmonicida*, homogeneous with no serotypes, which causes economically devastating losses in cultivated salmonids and non-salmonid fish in fresh andmarine waters. The oil-adjuvanted bacterin vaccine has been developed and commercialized since 1980 and still the main one for vaccinations of salmonids against *A. salmonicida* in commercial aquaculture[34,46,47]. Few different approaches have been done to develop live attenuated or recombinant vaccines against furunculosisbut not approved for commercialization yet (Table 7).

Table 7: DNA and live attenuated vaccines trials against *Aeromonas salmonicida*

Antigen	Adjuvant	Species	Route	RPS	Ref.
Recombinant A-layer protein	Alginate encapsulation	Goldfish	Oral	0	[48]
Live attenuated A-layer protein	-	Rainbow trout	imr	?	[49]
Live attenuated O-antigen	-	Rainbow trout	imr	?	[49]
Live attenuated aroA	-	Atlantic salmon	im	100	[50]

4. Motile Aeromonas septicaemia

Motile aeromonads of*A. hydrophila*, *A. sobria* and *A. cavieae* cause a haemorrhagic septicaemia innumerous species of cultured and wild marine-, brackish-, fresh-water fish. Outbreaks of Aeromonas septicaemia are usually related to change in environmental conditions such as handling stress, fish transfer, overcrowding, sudden change of temperature, low dissolved oxygen, poor nutritional status, and parasitic andfungal infections. Although several trials of vaccination of different fish species, the serological heterogeneity among the motile Aeromonas species render the development of a commercial vaccine (Table 8). Thepathogenesis of *A. hydrophila*is multi-factorial, and mediated by secretion of extracellular proteins such as aerolysin, lipase, chitinase, amylase, gelatinase, hemolysins, and enterotoxins[34,51].

Table 8: DNA and live attenuated vaccines trials against Motile Aeromonas septicaemia

Antigens	Adjuvant	Species	Route	RPS%	Ref.
Recombinant hemolysin co-regulated protein (Hcp) of the T6SS	?	Common carp	ip	46.67	[52]
Recombinant outer membrane protein R	modified herbal-oil adjuvant	Indian major carp	ip	33 CM	[53]
Bivalent A. veronii ompA and A. hydrophilahemolysins (hly) protein	PLGA (W/O/W) encapsulation.	Mice	ip		[54]
Recombinant Omp38	-	Chinese breams	ip	57.14	[55]
Recombinant multivalent WEDΔasdB/pUTta4DGap.*E. tarda*	-	Turbot	imr	94	[56]
Lipopolysaccharide LPS	-	Rainbowtrout	imr	34	[57]
Recombinant outer membrane Omp48	-	Rohu	im	69	[58]
Recombinant outer membrane adhesins (Aha1)	-	Common carp	ip	52	[59]
Recombinant outer membrane OMPW	-	Common carp	ip	71	[59]
Lipopolysaccharide LPS	-	Grass carp	ip	83.3	[60]
*V. anguillarum*empAand GAPDH from *A.hydrophila*	FCA	Turbot	ip	84	[10]
Subunit outer membrane proteins (OMP)	PLGAencapsulation.	Rohu	ip	**	[61]
Recombinant protein for the S-layer protein	Montanide	Common Carp	ip	56-87	[62]
Recombinant Omp-G from *A.hydrophila*	-	European eel	ip	50-70	[63]
Recombinant Omp-G from *A.sobria*		European eel	ip	75	[63]
Live attenuated *P. fluorescens*fur mutant	-	Japanese flounder	ip Oral imr	92.3 84.6 76.9	[20]
Live attenuated *P. fluorescens*fur mutant and pJAQ plasmid of *V. harveyi* (TFM/pJAQ)	-	Japanese flounder	ip	93	[20]
Recombinant outer membrane ompTS (37 kDa)	FCA	Indian major carp	ip	?	[64]
Subunitextracellular protease EprJ1	-	Mice	ip	60	[65]
Recombinant Aha1 adhesin	FCA	Blue gourami	ip	75-87.5	[13]
Liveattenuated AroA	-	Rainbow trout	ip	75	[66]

(CM) Cumulative mortalities, (**) Higher than other formulations

5. Pseudomonadiasis

The most Pseudomonas species isolated from diseased fish are *P. aeruginosa*, *P.anguilliseptica*, *P. fluorescens*, *P. putida*, and *P. plecoglossicida*. *P. fluorescens*is a commonaquaculture pathogen isolated in Egypt and worldwide that can infect a variety of farmed fishspecies including carp, tilapia, and catfish. Few DNA vaccines trials have been evaluated to protect fish against Pseudomonadiasis (Table 9).

Table 9: DNA and live attenuated vaccines trials against Pseudomonadiasis

Antigen	Adjuvant	Species	Route	RPS%	Ref.
Subunit *P. fluorescens* TonB-dependent outer membrane receptors (Tdr1, Tdr2, Tdr3)	-	Turbot			[67]
*P. putida*LPS	-	Large yellow croaker	ip	40	[68]
Subunit *P. fluorescens* TonB-dependent outer membrane receptors (TdrA)	Aluminum hydroxide	Japanese flounder	ip	80.6	[69]
Live attenuated *P. fluorescens*fur mutant	-	Japanese flounder	ip Oral imr	96.5 85.5 81.5	[20]

6. Yersiniosis

Y. ruckeri is the causative agent of enteric red mouth (ERM) disease, producing important economic losses in salmonid aquaculture worldwide. *Y. ruckeri* was also isolated from wild fish, birdsand mammals, and can dormant survive in the environment (seawater and sediments). *Y. ruckeri* vaccine was one of the first commercial fish vaccinedeveloped from serotype O1a with generallyhigh efficacy[34]. Table 10 showed new trials for developing vaccines in Rainbow trout based on *Y. ruckeri*virulence determinants.

Table 10: DNA and live attenuated vaccines trials against *Y. ruckeri*

Antigens	Adjuvant	Species	Routes	RPS (%)	Ref.
Lipopolysaccharide (LPS)	-	Rainbowtrout	ip	77.4-83.8	[70]
Recombinant flagellin protein of*Y. ruckeri* biotype 1 BA19	-	Rainbow trout	ip	68–72	[71]
Extracellular product of *Y. ruckeri*	-	Rainbow trout	imr	74–81.4	[72]
Live attenuated *Y. ruckeri*aroA gene	-	Rainbow trout	ip	90	[73]
Yrp1 protease toxoid of *Y. ruckeri*,strain 150RI4	-	Rainbow trout	ip	79	[74]

7. Edwardsiella tarda

E.tarda is an intracellular Gram-negative pathogen that causes edwardsiellosis, hemorrhagic septicemia, in fresh and marine fishclaimingsevere economic losses. *E.tarda*divided into four serotypes, A, B, C and D and can infect a broad range of hosts such as fish, birds, reptiles, amphibians, mammals, and humans. *E.tarda*harbor several virulence determinants; type III secretion system (T3SS), type VI secretionsystem (T6SS), adhesin and hemolysin[75]. Park *et al.*[76] reviewed the trails of vaccine development against *E. tarda*, whereas this review completed the other trails since 2012 (Table 11).

8. Edwardsiella ictaluri (Enteric septicaemia of catfish, ESC)

*E. ictaluri*isGram-negative, intracellular, flagellated bacteria, serologically homogeneous and phylogeneticallyrelated to Salmonella. *E. ictaluri*is one ofthe most prevalentdiseaseaffectingcultured channel catfish causing enteric septicemia of catfish (ESC) leading to huge economical loss in USA. Killed and live attenuated *E.ictaluri* vaccines have been developed tocontrol ESC. Several trial have been done to produce attenuated mutants using chemical/drug mutagenesis, transposon insertion and by auxotrophy. Currently, Klesius and Shoemaker [106] produced a live attenuated *E. ictaluri* rifampicin mutant from *E. ictaluri* EILO strain (Table 12). This modified live vaccine was found to be effective in controlling ESC in catfishand was registered as AquaVac-ESC in aquaculture by USDA to Intervet/Schering-Plough Animal Health[34].

9. Flavobacterium psychrophilum (Cold water disease)

F. psychrophilum, also called *Cytophaga psychrophila* or *Flexibacter psychrophilus*, is the cause of bacterial cold water disease or peduncledisease in salmonids. It has been isolated from rainbow trout fry syndrome, eel and ayu worldwide. Few vaccination attempts against*F. psychrophilum* have beenpublished because the bacterium is difficult to culture and isolate. Until now no commercial vaccine are available [34,112]. Some virulence factors have been described in *F. psychrophilum*pathogenesis such as extracellular proteases, iron acquisition system, adhesin, haemolytic activities, lipopolysaccharide (LPS) O-antigens, surface proteins and a thiol which considered perspective antigens for vaccine development (Table 13). However, the development of vaccine against *F. psychrophilum*is considered a difficult task[113].

Table 11: DNA and live attenuated vaccines trials against *E.tarda*

Antigen	Adjuvant	Species	Route	RPS	Ref.
Killed but metabolically active (KBMA)uvrA and uvrB genes knock-out E. tarda	-	Olive flounder	ip	100	[77]
Live attenuated ΔaroAΔesrB	-	Flounder	im imr	14.3-66.7 100	[78]
Live attenuated esrB	-	Turbot	ip imr	77.8 64.4	[75]
Recombinant GAPDH from E. ictaluri	ISA 763 AVG	Tilapia	ip	71.4	[79]
LiveattenuatedHfq (an RNA-binding protein)	-	Japanese flounder	imr	65-76	[80]
Recombinant multivalent WEDΔasdB/pUTta4DGap.*E. tarda*	-	Turbot	imr	83	[56]
Live attenuated aroA	-	Turbot	ip imr	NS	[81]
Bivalent porin Ⅱ of *A. hydrophila* and ompS2 of *E. tarda*	-	American Eels	ip	37.5	[82]
Live mutated in the T3SS genes for EseB, EseC, EseD and EscA, along with the aroC gene	-	Turbot	ip imr	73.3±3.3 63.3±3.3	[83]
recombinant subunit vaccineFimA	Aluminum hydroxide	Turbot	ip	71.9	[84]
Live attenuated vaccinetwin-arginine translocation (Tat)	-	Turbot	ip	81.81	[85]
Recombinant GAPDH	Montanide™ISA 763A	Turbot	ip	60	[86]
Recombinant vaccine DnaJ	Aluminum hydroxide	Olive flounder	ip	62	[87]
Recombinant vaccine OMP	-	Common carp	ip	54.3	[88]
Natural OMVs	-	Olive flounder	ip	70	[89]
Mutant alanine racemase (*alr*) gene and aspartatesemialdehyde dehydrogenase (asd)	-	Olive flounder	ip	100	[90]
Recombinant vaccine rEta2	-	Olive flounder	ip	83	[91]
DNA vaccine pCEta2	-	Olive flounder	im	67	[91]
Recombinant vaccine pCEsa1	-	Olive flounder	ip	57	[92]
Esa1-expressing recombinant strain	-	Olive flounder	po	52	[93]
Esa1-expressing recombinant strai	-	Olive flounder	ip	79	[93]
Live E22	-	Olive flounder	ip	45	[94]
DNA vaccine N163	-	Olive flounder	im	70.2	[95]
Recombinant vaccine scFv	FIA	Red drum	ip	88	[96]
Recombinant vaccine EseD	FIA	Turbot	ip	62.3	[97]
Recombinant vaccine DegPEt	FIA	Olive flounder	ip	89	[98]
Recombinant vaccine Et49	FIA	Olive flounder	ip	47	[98]
Recombinant vaccine Eta21	Bacillus sp. strain B187	Olive flounder	ip	69	[99]
DH5α/pTAET21	bacillus sp. strain B187	Olive flounder	ip	100	[99]
DNA vaccine pEta6		Olive flounder	im	50	[100]
Recombinant vaccine Eta6	Bacillus sp. strain B187	Olive flounder	ip	53	[100]
Recombinant vaccine Et18	Bacillus sp. strain B187	Olive flounder	ip	61	[101]
Recombinant vaccine EseD	Bacillus sp. strain B187	Olive flounder	ip	51	[101]
Live, attenuated esrB mutant		Turbot	ip	93.3	[102]
Ghost vaccine		Olive flounder	po	85.7	[103]
Ghost vaccine		Tilapia	ip	88.8	[104]
37 kDa OMP		Olive flounder	ip	70	[105]

(NS) Not significantly different from the control group

Table 12: DNA and live attenuated vaccines trials against *E. ictaluri*

Antigen	Adjuvant	Species	Route	RPS%	Ref.
Live attenuated tricarboxylic acid cycle (TCA) deletion	-	Catfish	imr	100	[107]
Live attenuated Novobiocin-resistant	-	Catfish	imrInjection	100 92–100	[108]
Live attenuated LPS deletionO side-chain	-	Catfish	imr Injection	0 94	[109]
Live attenuated aroA-deletion	-	Catfish	imr	54.1–63.8	[110]
Live attenuated rifampicin-resistant	-	Catfish	imr	60–100	[106]
Live attenuated purA-deletion	-	Catfish	imr	66.3	[111]

Table 13: DNA and live attenuated vaccines trials against *F. psychrophilum*

Antigen	Adjuvant	Species	Route	RPS%	Ref.
Recombinant CoA dehydrogenase (HCD)	-	Ayu	ip	36.8	[112]
RecombinantATP synthasebeta subunit (atpD)	-	Ayu	ip	31.5	[112]
Recombinantglutamate dehydrogenase (gdhA)	-	Ayu	ip	35.6	[112]
Recombinant subunit *rpoB*	FCA	Rainbow trout	ip	NS	[114]
Recombinant factor-Tu, SufB and Fe-S	-	Rainbow trout	ip	NS	[115]
Recombinant DNA heat shock proteins (Hsp) 60, 70	FCA	Rainbow trout	ip	NS	[116]
Live attenuated ExbD2	-	Rainbow trout	im	81.8	[117]
Low molecular mass fraction (P25-33)	FCA	Rainbow trout	ip	10-15 CPM	[118]
Recombinant ribosomal protein L10	FCA	Rainbow trout	ip	82	[119]
OmpA protein	FCA	Rainbow trout	ip	-	[120]
OmpH-like protein	FCA	Rainbow trout	ip	88.5	[121]
Outer membrane fraction (OMF)	-	Ayu	ip	80-85	[122]

NS: no significance difference between control and vaccine
CPM: mean cumulative percent mortality.

10. Flavobacterium columnare (Columnaris disease or saddleback disease)

F. columnare,(syn., *Chondrococcus columnaris*, *Cytophaga columnaris*, *Flexibacter columnaris*), is Gram-negative chromogenic glidingbacterial pathogen associated with columnaris diseasein several freshand brackish water fish species worldwide. Several vaccination experiments with formalin killedbacterins with and without adjuvants against *F. columnare* have been performed and resulted in low protection (Table 14). In 2005, an attenuated live vaccine against columnaris disease was developed by repeated passage of a virulent strain on rifampicin and been licensed byIntervet/Schering-Plough Animal Health for use in channel catfishandlargemouth bass fry[123,124].

Table 14: DNA and live attenuated vaccines trials against *F. columnare*

Antigen	Adjuvent	Species	Route	RPS	Ref.
F. columnare ghosts by PhiX174lysis gene *E*	-	Grass carp	ip	70.9	[124]
Live attenuated rifampicin LPS mutated	-	Channel catfish	imr	57-96.4	[123]
Live attenuated rifampicin LPS mutated	-	Largemouth bass	imr	57-96.4	[123]
Recombinant heat shock protein (HSP) gene dna J	FCA	Channel catfish	ip	23*	[125]

(*) Lower than control group

11. Streptococcus iniae

S. iniae is Gram-positive bacterial organism has emerged as an important aquaticpathogen responsible for invasivedisease outbreaks incultured fish around the world. Streptococcosis can lead to severe symptoms withhigh mortality rates and substantialeconomic losses in tilapia, hybrids striped bassand rainbow trout. *S. iniae* is also been identified as a potentialzoonotic pathogencause softtissue infections after handling raw fish. Most of vaccines attempts against streptococcosis showed good levels of protection especially with intraperitoneal injection. *S. iniae* has potential virulence determinants such as capsule, M-like protein, phosphoglucomutase, streptolysin S, sivS/R, CpsY, GAPDHand Sortase A which used in vaccination trials and gave different protection rates [34,126,127] as shown in Table 15.

Table 15: DNA and live attenuated vaccines trials against *S. iniae*

Antigen	Adjuvent	Species	Route	RPS	Ref.
Recombinant Enolase (ENO)	-	Zebrafish	ip	100	[128]
Live attenuated Sortase A(srtA)	-	Nile tilapia	ip	95.5	[126]
DNA monovalent streptolysin S cluster sagF, SagG, or SagI	-	Japanese flounder	im	65-78	[129]
DNA divalent streptolysin S cluster				4-17	
DNA multivalent streptolysin S cluster				13-26	
Live attenuated novobiocin-resistant	-	Nile tilapia	ip	75-100	[130]
Recombinant iron-binding protein (Sip11)	Bacillus sp. B187	Japanese flounder	ip	69.7	[131]
DNA secretory antigen, Sia10	?	Turbot	?	73.9-92.3	[132]
Recombinant GAPDH Ghost	-	Olive flounder	oral	57 CM	[133]
Liveattenuated M-likeprotein (Delta simA)	-	Hybrid striped bass	ip	100	[134]
Phosphoglucomutase (*pgm*) gene	-	Hybrid striped bass	ip	90-100	[127]

CM: cumulative mortalities

12. Lactococcus garvieae

L. garvieae is a septicemic gram-positive bacterium infecting several species of marine and fresh water fish and mammals. The injectable vaccine amberjack/yellowtailhas been licensed since 2000 in Japan. Also, the commercial vaccines are available for rainbow trout in France, Italy, and UK. The *L. garvieae* bacterins displayed excellent effectiveness and high levels of long-termprotection[47].

Also, inactivated autovaccines have also been developed from outbreaks with the causative strains of *L. garvieae*[135]. live attenuated *L. garvieae* as an experimental vaccine has alsobeen reported. An attenuated *L. garvieae* strain lacking a virulence-associated capsule on itscell surface as a live vaccine has been reported to confer long-lasting protection to yellowtail, *Seriola quinqueradiata* [136]. A trial for using recombinant subunit vaccine of 40 kDa GAPDH of *L. garvieae*adjuvant with ISA to protect tilapia. The relative survival rate of the immunized fish with GAPDH+ISA after challenged was 50% [137].

Conclusion

- DNA vaccine has several advantages over conventional vaccines and have been increasingly employed against many of the fish pathogens depending on bacterial virulence determinants. It is expected that some more genetically modified vaccines may be commercialized in the near future.
- The development of effective vaccines should be accompanied with the application of specific adjuvants that maximise the immunogenicity of the vaccine. Adjuvants such as the TLR ligands or cytokines showed promising results. In addition, nanoparticles found their way in vaccine delivery and encapsulation.
- Development of polyvalent vaccines is crucial due to a wide variety of bacterial infections in aquaculture. Moreover, polyvalent vaccine could play a role in development of effective vaccine(s) to overcome the heterogeneity of motile aeromon as septicemia and intracellular parasitism of *E. tarda.*

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