Bacterial Fish Pathogens

Diseases of Farmed and Wild Fish

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Fourth Edition

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Preface

This fourth edition of *Bacterial Fish Pathogens* is the successor to the original version, first published by Ellis Horwood Limited in 1987, and was planned to fill the need for an up-to-date comprehensive text on the biological aspects of the bacterial taxa which cause disease in fish. The impetus to prepare a fourth edition stemmed initially from discussion with Chinese colleagues when it became apparent that the book was particularly well used and cited (>1,600 citations in China since 1999). Since publishing the third edition, there has been a slowing down in the number of new fish pathogens. However, there has been a steady increase in the number of publications about some aspects of bacterial fish pathogens, including the application of molecular techniques to diagnosis and pathogenicity studies. Consequently, we considered that it is timely to consider the new information in a new edition. The task was made immeasurably easier by the ready availability of electronic journals, which could be accessed from the office. Weeks of waiting for inter-library loans did not feature during the research phase of the project. Our strategy was to include information on new pathogens and new developments on well-established pathogens, such as *Aeromonas salmonicida* and *Vibrio anguillarum*. Because of the deluge of new information, we have needed to be selective, and in particular, we have once again condensed details of the pathology of the diseases, because there are excellent texts already available that cover detailed aspects of the pathological conditions. Nevertheless, this fourth edition will hopefully meet the needs of the readership. As with all the preceding editions, it is emphasised that most of the information still appertains to diseases of farmed, rather than wild, fish.

The scope of the book covers all of the bacterial taxa that have at one time or another been reported as fish pathogens. Of course, it is realised that some taxa are merely secondary invaders of already damaged tissues, whereas others comprise serious, primary pathogens. Shortcomings in the literature or gaps in the overall understanding of the subject have been highlighted.

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Abbreviations and acronyms

Aer.	*Aeromonas*
AFLP	Amplified Fragment Length Polymorphism
AHL	Acylated Homoserine Lactone
A-layer	The additional surface layer of *Aer. salmonicida*
Arc.	*Arcobacter*
ARISA	Automated Ribosome Intergenic Spacer Analysis
ATCC	American Type Culture Collection, Rockville, Maryland
BHI	Brain Heart Infusion
BHIA	Brain Heart Infusion Agar
BKD	Bacterial Kidney Disease
BLIS	Bacteriocin-Like Substance
BMA	Basal Marine Agar
bp	base pair
Car.	*Carnobacterium*
CBB	Coomassie Brilliant Blue agar
CDC	Centers for Disease Control and Prevention, Atlanta, Georgia
CE	Carp Erythrodermatitis
CFU	Colony-Forming Unit
CgP	Cytidine-phosphate-Guanosine
Chrys.	*Chryseobacterium*
CHSE-214	CHinook Salmon Embryo 214 cell line
Cit.	*Citrobacter*
Cl.	*Clostridium*
CLB	*Cytophaga*-Like Bacteria
CLED	Cystine Lactose Electrolyte-Deficient agar
Cor.	*Corynebacterium*
CpG	Cytidine-phosphate-Guanosine

Cyt.	*Cytophaga*
DNA	DeoxyriboNucleic Acid
ECP	ExtraCellular Product
EDTA	Ethylene Diamine Tetraacetic Acid
Edw.	*Edwardsiella*
ELISA	Enzyme-Linked ImmunoSorbent Assay
En.	*Enterococcus*
Ent.	*Enterobacter*
EPC	Epithelioma Papulosum Cyprini (cell line)
ERM	Enteric RedMouth
Esch.	*Escherichia*
Eu.	*Eubacterium*
FAME	Fatty Acid Methyl Ester
FAT	Fluorescent Antibody Test
FCA	Freund's Complete Adjuvant
FIA	Freund's Incomplete Adjuvant
Fla.	*Flavobacterium*
Fle.	*Flexibacter*
G + C	Guanine plus Cytosine
GCAT	Glycerophospholipid : Cholesterol AcylTransferase
GFP	Green Fluorescent Protein
GMD	Glucose Motility Deeps
H.	*Haemophilus*
Haf.	*Hafnia*
HG	Hybridisation Group
hsp	heat shock protein
i.m.	intramuscular
i.p.	intraperitoneal
iFAT	indirect Fluorescent Antibody Test
IROMP	Iron-Regulated Outer Membrane Protein
ISR	Intergenic Spacer Region
IU	International unit
J.	*Janthinobacterium*
kb	kilobase
kDa	kiloDalton
KDM2	Kidney Disease Medium 2
LAMP	Loop-mediated isothermal AMPlification
LD_{100}	Lethal Dose 100%
LD_{50}	Lethal Dose 50%, i.e. the dose needed to kill 50% of the population
Lis.	*Listeria*
LPS	LipoPolySaccharide
MDa	megaDalton
MHC	Mueller–Hinton agar supplemented with 0.1% (w/v) L-cysteine hydrochloride

MIC	Minimum Inhibitory Concentration
MIS	Microbial Identification System
Mor.	*Moraxella*
mRNA	messenger RNA
MRVP	Methyl Red Voges Proskauer
msa	major soluble antigen (gene)
MSS	Marine Salts Solution
Myc.	*Mycobacterium*
NCBV	Non-Culturable But Viable
NCIMB	National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland
Nec.	*Necromonas*
Noc.	*Nocardia*
ODN	OligoDeoxyNucleotide
OMP	Outer Membrane Protein
ORF	Open Reading Frame
p57	57 kDa protein (of *Ren. salmoninarum*)
Pa.	*Pasteurella*
PAGE	PolyAcrylamide Gel Electrophoresis
PAP	Peroxidase–AntiPeroxidase enzyme immunoassay
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
PFU	Plaque Forming Unit
Ph.	*Photobacterium*
PMSF	PhenylMethyl–Sulphonyl Fluoride
Pr.	*Providencia*
Ps.	*Pseudomonas*
QPCR	Quantitative Polymerase Chain Reaction
RAPD	Randomly Amplified Polymorphic DNA
Ren.	*Renibacterium*
RFLP	Restriction Fragment Length Polymorphism
RLO	*Rickettsia*-Like Organisms
ROS	Reactive Oxygen Species
RPS	Relative Percent Survival
rRNA	ribosomal RiboNucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RTFS	Rainbow Trout Fry Syndrome
RTG-2	Rainbow Trout Gonad-2 cell line
Sal.	*Salmonella*
SBL	Striped Bass Larvae
S_D	Dice coefficient
S-layer	Surface layer
SDS	Sodium Dodecyl Sulphate
Ser.	*Serratia*

SKDM	Selective Kidney Disease Medium
SSH	Suppression Subtractive Hybridisation
Sta.	*Staphylococcus*
Str.	*Streptococcus*
TCBS	Thiosulphate Citrate Bile Salts Sucrose Agar
TCID	Tissue Culture Infectivity Dose
TSA	Tryptone Soya Agar
TSB	Tryptone Soya Broth
V.	*Vibrio*
Vag.	*Vagococcus*
VAM	*Vibrio Anguillarum* Medium
vapA	virulence array protein gene A
VHH	*Vibrio harveyi* Haemolysin
VHML	*Vibrio harveyi* Myovirus-Like (bacteriophage)
Y.	*Yersinia*

About the authors

Brian Austin is Dean of the University (Science and Engineering) and Professor of Microbiology in the School of Life Sciences, Heriot-Watt University. From 1975 to 1978 he was Research Associate at the University of Maryland, U.S.A., and from 1978 to 1984 he was Head of Bacteriology at the Fish Diseases Laboratory in Weymouth, U.K. He joined Heriot-Watt University as a Lecturer in Aquatic Microbiology in 1984.

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1

Introduction

Representatives of many bacterial taxa have, at one time or another, been associated with fish diseases. However, not all of these bacteria constitute primary pathogens. Many should be categorised as opportunistic pathogens, which colonise and cause disease in already damaged hosts. Here, the initial weakening process may involve pollution or a natural physiological state (e.g. during the reproductive phase) in the life cycle of the fish. There remains doubt about whether some bacteria should be considered as fish pathogens. In such cases, the supportive evidence is weak or non-existent. Possibly, such organisms constitute contaminants or even innocent saprophytes. However, it is readily apparent that there is great confusion about the precise meaning of disease. A definition, from the medical literature, states that:

> "... a disease is the sum of the abnormal phenomena displayed by a group of living organisms in association with a specified common characteristic or set of characteristics by which they differ from the norm of their species in such a way as to place them at a biological disadvantage ..."
>
> (Campbell *et al.*, 1979)

This definition is certainly complex, and the average reader may be excused for being only a little wiser about its actual meaning. Dictionary definitions of disease are more concise, and include "an unhealthy condition" and "infection with a pathogen [= something that causes a disease]". One conclusion is that disease is a complex phenomenon, leading to some form of measurable damage to the host. Yet, it is anticipated that there might be profound differences between scientists about just what constitutes a disease. Fortunately, infection by micro-organisms is one aspect of disease that finds ready acceptance within the general category of disease.

For his detailed treatise on diseases of marine animals, Kinne (1980) considered that disease may be caused by:

- genetic disorders;
- physical injury;
- nutritional imbalance;
- pathogens;
- pollution.

This list of possible causes illustrates the complexity of disease. An initial conclusion is that disease may result from biological (= *biotic*) factors, such as pathogens, and *abiotic* causes, e.g. the emotive issue of pollution. Disease may also be categorised in terms of epizootiology (Kinne, 1980), namely as:

- *Sporadic* diseases, which occur sporadically in comparatively small numbers of a fish population.
- *Epizootics*, which are large-scale outbreaks of communicable disease occurring temporarily in limited geographical areas.
- *Panzootics*, which are large-scale outbreaks of communicable disease occurring over large geographical areas.
- *Enzootics*, which are diseases persisting or re-occurring as low-level outbreaks in certain areas.

The study of fish diseases has concentrated on problems in fish farms (= aquaculture), where outbreaks either begin suddenly, progress rapidly often with high mortalities, and disappear with equal rapidity (= *acute* disease) or develop more slowly with less severity, but persist for greater periods (= *chronic* disease).

This text will deal with the diseases caused by bacteria. However, it is relevant to emphasise that disease is not necessarily caused by single bacterial taxa. Instead, there may well be synergistic interactions between two or more taxa. This possibility is often ignored by scientists. Then, there are the situations in which infectious diseases are suspected but not proven. An example includes red mark syndrome/disease (also known as winter strawberry disease) of rainbow trout in the U.K. where the causal agent is suspected—but not proven—to be bacterial of which *Fla. psychrophilum* or *Aer. hydrophila* are suspected to be the possible aetiological agent.

Disease is usually the outcome of an interaction between the host (= fish), the disease-causing situation (= pathogen) and external stressor(s) (= unsuitable changes in the environment; poor hygiene; stress). Before the occurrence of clinical signs of disease, there may be demonstrable damage to/weakening of the host. Yet all too often, the isolation of bacteria from an obviously diseased fish is taken as evidence of infection. Koch's Postulates may be conveniently forgotten.

So, what are the bacterial fish pathogens? A comprehensive list of all the bacteria, which have been considered to represent fish pathogens, has been included in Table 1.1 (see p. 4). Some genera, e.g. *Vibrio*, include many species that are acknowledged to be pathogens of freshwater and/or marine fish species. Taxa (highlighted by quotation marks), namely "*Catenabacterium*", "*H. piscium*" and "*Myxobacterium*" are of doubtful taxonomic validity. Others, such as *Pr. rettgeri* and *Sta. epidermidis*, are of questionable significance in fish pathology insofar as their recovery from diseased

animals has been sporadic. A heretical view would be that enteric bacteria (e.g., *Providencia*), comprise contaminants from water or from the gastro-intestinal tract of aquatic or terrestrial animals. Many of the bacterial pathogens are members of the normal microflora of water and/or fish. Others have been associated only with clinically diseased or covertly infected (asymptomatic) fish. Examples of these "obligate" pathogens include *Aer. salmonicida* and *Ren. salmoninarum*, the causal agents of furunculosis and bacterial kidney disease (BKD), respectively. In later chapters, it will be questioned whether or not bacteria should be considered as obligate pathogens of fish, at all. It is a personal view that the inability to isolate an organism from the aquatic environment may well reflect inadequate recovery procedures. Could the organism be dormant/damaged/senescent in the aquatic ecosystem; a concept which has been put forward for other water-borne organisms (Stevenson, 1978)?

It is undesirable that any commercially important species should suffer the problems of disease. Unfortunately, the aetiology of bacterial diseases in the wild is often improperly understood. Moreover, it seems that little if anything may be done to aid wild fish stocks, except, perhaps, by controlling pollution of the rivers and seas, assuming that when environmental quality deteriorates this influences disease cycles. In contrast, much effort has been devoted to controlling diseases of farmed fish.

Conclusion

- The list of fish pathogens has extended substantially since 1980. Current interest focuses on the vibrios, CLBs, mycobacteria and streptococci–lactococci.
- A question mark hangs over the significance of some organisms to fish pathology—are they truly pathogens or chance contaminants?
- There has been considerable improvement in the taxonomy of some groups (e.g., vibrios).
- There has been a shift from emphasis on culture-dependent to culture-independent techniques.
- Molecular methods have become commonplace in laboratories involved in the study of fish diseases.

Table 1.1. Bacterial pathogens of freshwater and marine fish

Pathogen	Disease	Host range	Geographical distribution
ANAEROBES			
"*Catenabacterium*" sp.	—	Grey mullet (*Mugil auratus*)	U.S.A.
Clostridiaceae representative			
Clostridium botulinum	Botulism	Redfish (*Sebastes* sp.) Salmonids	Denmark, England, U.S.A.
Eubacteriaceae representative			
Eubacterium tarantellae	Eubacterial meningitis	Striped mullet (*Mugil cephalus*)	U.S.A.
GRAM-POSITIVE BACTERIA— THE "LACTIC ACID" BACTERIA			
Carnobacteriaceae representative			
Carnobacterium piscicola	Lactobacillosis, pseudokidney disease	Salmonids	North America, U.K.
Enterococcaceae representatives			
Enterococcus (Streptococcus) faecalis subsp. *liquefaciens*	—	Rainbow trout (*Oncorhynchus mykiss*), catfish	Italy
Vagococcus salmoninarum	Lactobacillosis, pseudokidney disease, peritonitis, septicaemia	Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), rainbow trout	Australia, France, North America
Lactobacillaceae representative			
Lactobacillus spp.	Lactobacillosis, pseudokidney disease	Salmonids	North America, U.K.
Streptococcaceae representatives			
Lactococcus garvieae (= Enterococcus seriolicida)	Streptococcicosis/ streptococcosis	Many fish species	Australia, Europe, Israel, Japan, Saudi Arabia, Red Sea, South Africa, Taiwan, U.S.A.

Lactococcus piscium	Lactobacillosis, pseudokidney disease	Rainbow trout	North America
Streptococcus dysgalactiae	—	Amberjack (*Seriola dumerili*), yellowtail (*Seriola quinqueradiata*)	Japan
Streptococcus difficilis (= Str. agalactiae)	Meningo-encephalitis	Carp (*Cyprinus carpio*), rainbow trout, silver pomfret (*Pampus argenteus*), tilapia (*Oreochromis* spp.)	Israel, Kuwait, USA
Streptococcus iniae (= Str. shiloi)	Meningo-encephalitis, streptococcicosis/ streptococcosis	Various freshwater and coastal fish species	Australia, Bahrain, Europe, Israel, Japan, Saudi Arabia, South Africa, U.S.A.
Streptococcus milleri	—	Koi carp (*Cyprinus carpio*)	U.K.
Streptococcus parauberis	Streptococcicosis/ streptococcosis	Turbot (*Scophthalmus maximus*)	Spain
AEROBIC GRAM-POSITIVE RODS AND COCCI			
Renibacterium salmoninarum	Bacterial kidney disease (BKD; Dee disease; corynebacterial kidney disease)	Salmonids	Europe, Japan, North and South America
Bacillaceae representatives			
Bacillus spp.	Septicaemia; bacillary necrosis	Various freshwater fish species including catfish (*Pangasius hypophthalmus*)	Nigeria, Vietnam
Bacillus cereus	Branchio-necrosis	Carp (*Cyprinus* sp.), striped bass (*Morone saxatilis*)	U.S.A.
Bacillus mycoides	Ulceration	Channel catfish (*Ictalurus punctatus*)	Poland, U.S.A.
Bacillus subtilis	Branchio-necrosis	Carp	Poland

(*continued*)

Table 1.1 (*cont.*)

Pathogen	Disease	Host range	Geographical distribution
Corynebacteriaceae representatives			
Corynebacterium aquaticum	Exophthalmia	Striped bass	U.S.A.
Coryneform bacteria	"Corynebacteriosis"	Salmonids	England
Micrococcaceae representative			
Micrococcus luteus	Micrococcosis	Rainbow trout	England
Mycobacteriaceae representatives			
Mycobacterium spp. (*Myc. abscessus, Myc. anabanti, Myc. chelonei* subsp. *piscarium, Myc. fortuitum, Myc. gordonae, Myc. marinum, Myc. montefiorense, Myc. neoaurum, "Myc. piscium", "Myc. platypoecilus", Myc. pseudoshottsii, "Myc. ranae", "Myc. salmoniphilum", Myc. shottsii, Myc. scrofulaceum, Myc. simiae, Myc. smegmatis, Myc. ulcerans*	Mycobacteriosis (fish tuberculosis)	Most fish species	Worldwide
Nocardiaceae representatives			
Nocardia spp. (*Noc. asteroides, Noc. salmonicida, Noc. seriolae*)	Nocardiosis	Most fish species	Worldwide
Rhodococcus sp.	Ocular oedema	Chinook salmon (*O. tshawytscha*)	Canada
Rhodococcus erythropolis	?	Atlantic salmon	Norway, Scotland
Planococcaceae representative			
Planococcus sp.	—	Salmonids	England

Staphylococcaceae representatives			
Staphylococcus aureus	Eye disease	Silver carp (*Hypophthalmichthys molitrix*)	India
Staphylococcus epidermidis	—	Gilthead sea bream (*Sparus aurata*), red sea bream (*Chrysophrus major*), yellowtail (*Seriola quinqueradiata*)	Japan, Turkey
Staphylococcus warneri	Ulcerations	Rainbow trout	Spain
GRAM-NEGATIVE BACTERIA			
Aeromonadaceae representatives			
Aeromonas allosaccharophila	—	Elvers	Spain
Aeromonas bestiarum	—		U.S.A.
Aeromonas caviae	Septicaemia	Atlantic salmon (*Salmo salar*)	Turkey
Aeromonas hydrophila (= Aer. liquefaciens, Aer. punctata)	Haemorrhagic septicaemia, motile aeromonas septicaemia, redsore disease, fin rot	Many freshwater fish species	Worldwide
Aeromonas jandaei	—	Eel (*Anguilla* sp.)	Spain
Aeromonas salmonicida (subspecies *achromogenes, masoucida, salmonicida and smithia*) {= *Haemophilus piscium*}	Furunculosis, carp erythrodermatitis, ulcer disease	Salmonids, cyprinids, and marine species (dabs, cod)	Worldwide
Aeromonas sobria	—	Perch (*Perca fluvialitis*), gizzard shad (*Dorosoma cepedianum*)	Switzerland, U.S.A.
Aeromonas veronii biovar sobria	Epizootic ulcerative syndrome	African catfish (*Clarias gariepinus*), rajputi (*Puntius gonionotus*), rui (*Labeo rohita*), catla (*Catla catla*), shole (*Channa striatus*)	Bangladesh

(*continued*)