

## Strawberry disease lesions in rainbow trout from southern Idaho are associated with DNA from a *Rickettsia*-like organism

Sonja J. Lloyd<sup>1</sup>, Scott E. LaPatra<sup>1,2</sup>, Kevin R. Snekvik<sup>1,3</sup>, Sophie St-Hilaire<sup>4</sup>, Kenneth D. Cain<sup>5</sup>, Douglas R. Call<sup>1,\*</sup>

<sup>1</sup>Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164-7040, USA

<sup>2</sup>Clear Springs Foods Inc., PO Box 712, Buhl, Idaho 83316, USA

<sup>3</sup>Washington Animal Disease Diagnostic Laboratory, PO Box 647034, Pullman, Washington 99164-7034, USA

<sup>4</sup>Department of Biological Sciences, Idaho State University, 921 South 8th Avenue, Stop 8007, Pocatello, Idaho 83209-8007, USA

<sup>5</sup>Department of Fish and Wildlife Resources, University of Idaho, Moscow, Idaho 83844-1136, USA

\*Corresponding author. Email: drcall@wsu.edu

*Diseases of Aquatic Organisms* 82:111–118 (2008)

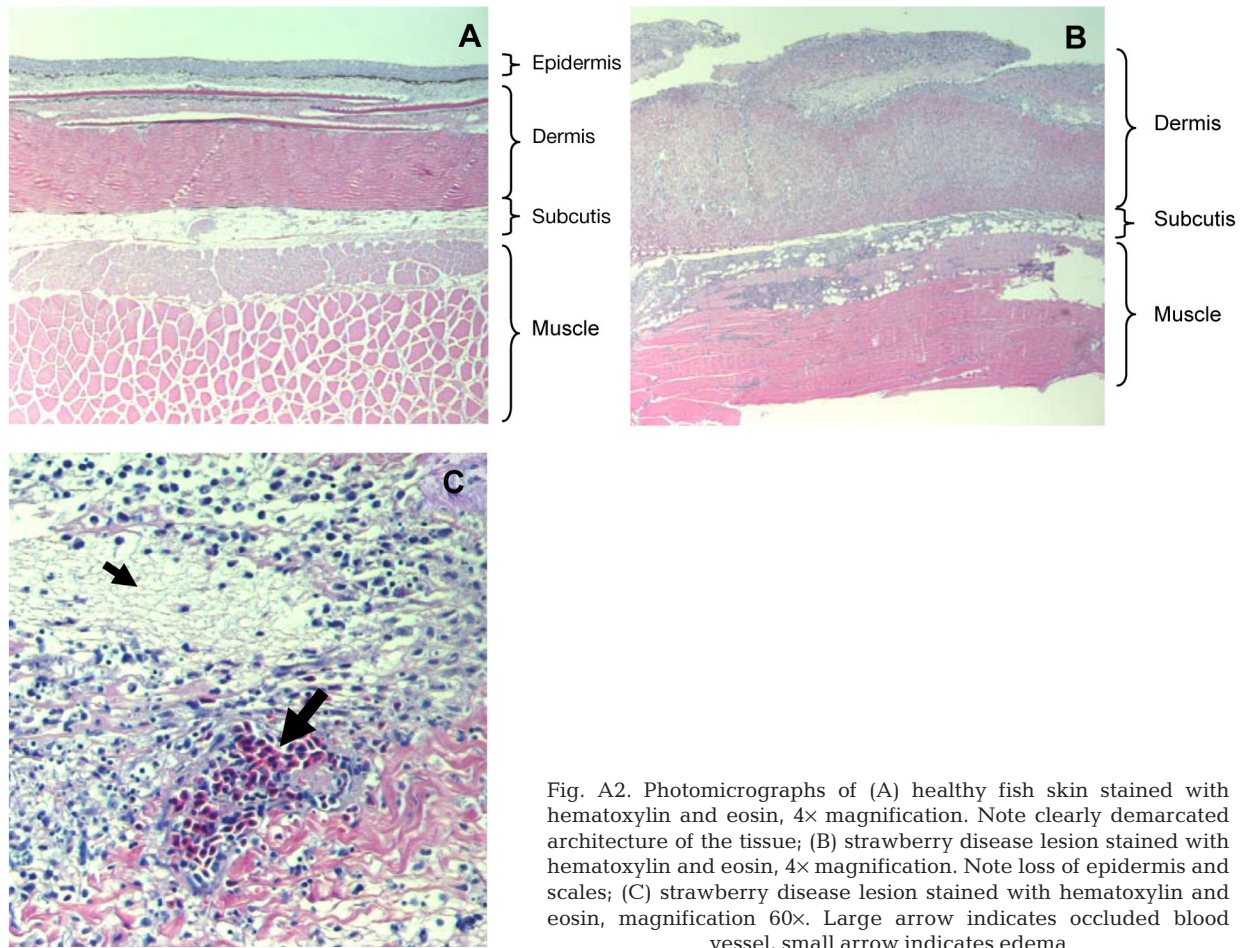
### Appendix 1. Case definition of strawberry disease lesions

Strawberry disease (SD) lesions are found on random locations on the trunk and generally do not affect fins or the head. Early SD lesions are first evident as small round foci with a red center characterized by slight lifting of the scales, whereas established SD lesions are thickened bright red lesions that typically have scale loss and may have a central area of necrosis (Fig. A1). Microscopically, these lesions consist of a locally severe ulcerative dermatitis characterized by moderate to large numbers of lymphocytes and macrophages admixed with abundant accumulations of edema fluid within the stratum spongiosum and stratum compactum (Fig. A2). The areas of inflammation are transected by frequent small blood vessels lined with reactive endothelial cells (neovascularization) and are occasionally disrupted by minimal to

locally extensive foci of necrosis that obliterate the normal architecture. The tunica media of scattered superficial dermal blood vessels is disrupted by accumulations of fibrin and edema fluid interspersed with degenerative to pyknotic inflammatory cells. Some of these vessels are partially to completely thrombosed by accumulations of fibrin and cellular debris. In the most severely affected lesions, the above inflammatory changes within the dermis extend through and obliterate the subcutis and multifocally infiltrate the skeletal muscle. In areas in which the skeletal muscle is disrupted by the inflammation, the myocytes range from degenerate with fragmentation and vacuolation of the sarcoplasm to necrotic with contracted cellular borders, hypereosinophilic sarcoplasm and pyknotic nuclei.



Fig. A1. A representative strawberry disease lesion presenting on a rainbow trout *Oncorhynchus mykiss* collected in southern Idaho



**Appendix 2.** Table A1. Accession numbers, descriptions and counts of 16S rDNA sequences recovered from strawberry disease (SD) lesion and healthy skin libraries

Accession	Description	Lesion	Healthy
AB015518.1	Unidentified proteobacterium	1 <sup>b</sup>	0
AB039334.1	<i>Brevibacillus agri</i>	1	0
AB059480.1	<i>Clostridium</i> sp.	0	4
AB066340.1	<i>Brevibacterium</i> sp.	1	0
AB075683.1	<i>Enterococcus</i> sp.	1	0
AB111104.1	<i>Proteobacterium</i>	0	1
AB128872.1	Uncultured bacterium	14 <sup>a</sup>	5 <sup>a</sup>
AB166881.1	<i>Phenylobacterium koreense</i>	1	0
AB177316.1	Uncultured bacterium	2	0
AB195776.1	Aquatic bacterium	3	0
AB240505.1	Uncultured bacterium	3	0
AB255115.1	Uncultured bacterium	3	9
AB294318.1	Uncultured bacterium	2 <sup>b</sup>	0
AF145257.1	<i>Corynebacterium xerosis</i>	1	0
AF205140.1	<i>Ehrlichia</i> sp. 'HGE agent'	1 <sup>b</sup>	0
AF276640.1	<i>Corynebacterium</i> sp.	2	0
AF385525.1	<i>Streptococcus</i> sp.	3	0
AF408936.1	<i>Pseudomonas</i> sp.	1	0
AF427039.2	<i>Pseudoxanthomonas taiwanensis</i>	1	0
AF451251.1	<i>Acinetobacter</i> sp.	4	6
AF513962.1	Uncultured <i>Propionibacterineae</i> bacterium	15	4
AF525481.1	<i>Rickettsiales</i> bacterium It62	92	0
AF525482.1	<i>Rickettsiales</i> bacterium It86	2	0
AF539679.1	<i>Acinetobacter</i> sp.	4	1
AJ269515.1	<i>Moraxella osloensis</i>	1	0
AJ295562.1	Uncultured rape rhizosphere bacterium	0	1
AJ310412.1	<i>Subtercola pratensis</i>	2	0
AJ313027.1	<i>Brevibacillus</i> sp.	2	0
AJ489328.1	<i>Geobacillus thermoleovorans</i>	1	0
AJ548899.1	Uncultured bacterium	3	3
AJ550464	<i>Bacillus silvestris</i>	14 <sup>a</sup>	0
AJ575540.1	Uncultured actinobacterium	1	0
AJ622907.1	<i>Kocuria carniphila</i>	2	0
AJ717350.1	<i>Agrococcus jenensis</i>	1	0
AJ786020.1	<i>Bacteroidetes</i> bacterium	1	0
AJ871304.1	<i>Modestobacter versicolor</i>	1	0
AM111056.1	<i>Arthrobacter</i> sp.	1	0
AM159183.2	<i>Chryseobacterium hispanicum</i>	0	1
AM159306.1	Uncultured <i>Clostridiaceae</i> bacterium	0	2
AM184302.1	<i>Comamonas</i> sp.	1	2
AM230493.1	<i>Flavobacterium succinicans</i>	0	1
AM237384.1	<i>Pedobacter cryoconitis</i>	1	0
AM279215.1	<i>Pedobacter soli</i>	1	0
AM285013.1	<i>Variovorax</i> sp.	2	0
AM423086.1	<i>Chryseobacterium hominis</i>	0	1
AM690925.1	Uncultured actinobacterium	7	0
AM697118.1	Uncultured bacterium	1	0
AM697234.1	Uncultured bacterium	1	0
AM697409.1	Uncultured bacterium	1	0
AM697491.1	Uncultured bacterium	0	1 <sup>b</sup>
AM711590.1	<i>Sphingomonas</i> sp.	1	0
AY064412.2	<i>Rhizobium</i> sp.	3	0
AY095437.1	Uncultured yard-trimming-compost bacterium	3	0
AY173079.1	<i>Streptococcus bovis</i>	1	0
AY176770.1	<i>Acinetobacter lwoffii</i>	1	0
AY193101.1	Uncultured proteobacterium	1	0
AY211144.1	<i>Microbacterium barkeri</i>	9	0
AY214753.1	Uncultured candidate division OP11 bacterium	5 <sup>b</sup>	0

Appendix 2. Table A1 (continued)

AY250098.1	Uncultured bacterium	1	0
AY258065.1	<i>Acidovorax</i> sp.	2	0
AY268331.1	Uncultured bacterium	1	0
AY297809.1	Betaproteobacterium	5	0
AY332197.1	<i>Bacillus</i> sp.	1	0
AY345531.1	Bacterium W20	1	0
AY349412.1	<i>Sphingomonas</i> sp.	1	0
AY437440.1	Uncultured <i>Bradyrhizobium</i> sp.	1	0
AY444817.1	<i>Bacteroidetes</i> bacterium	2	0
AY456700.1	<i>Pseudomonas</i> sp.	1	0
AY494684.1	Uncultured <i>Bacteroidetes</i> bacterium	2	0
AY504457.1	<i>Paenibacillaceae</i> bacterium	1	0
AY527757.1	Uncultured bacterium	1	0
AY559415.1	Uncultured bacterium	1	0
AY568513.2	<i>Bradyrhizobium elkanii</i>	2	0
AY594193.1	<i>Tepidimonas arfidensis</i>	0	1
AY632569.1	<i>Geobacillus stearothermophilus</i>	2	0
AY661998.1	Uncultured bacterium	0	1
AY662494.1	<i>Flavobacterium psychrophilum</i> strain CSF 259-93	2	0
AY770721.1	Uncultured gammaproteobacterium from sea squirt	1	0
AY881680.1	Uncultured <i>Acinetobacter</i> sp.	0	1
AY898005.1	Uncultured organism	1	0
AY907742.1	Uncultured bacterium	1	0
AY947925.1	Uncultured betaproteobacterium	2	0
AY947969.1	Uncultured <i>Bacteroidetes</i> bacterium	1	0
AY948064.1	Uncultured alphaproteobacterium	1	0
AY957950.1	Uncultured bacterium	1	0
AY958993.1	Uncultured bacterium	1	0
AY959164.1	Uncultured bacterium	0	1
AY960261.1	Uncultured <i>Pseudomonadaceae</i> bacterium	1	0
AY960264.1	Uncultured <i>Enterobacteriaceae</i> bacterium	2	0
AY960266.1	Uncultured <i>Enterobacteriaceae</i> bacterium	1	0
AY960268.1	Uncultured <i>Enterobacteriaceae</i> bacterium	0	7
AY962272.1	Uncultured bacterium	1	0
AY963424.1	Uncultured bacterium	1	0
AY972175.1	<i>Pseudomonas putida</i>	0	1
AY987770.1	<i>Aeromonas</i> sp.	2	0
CP000312.1	<i>Clostridium perfringens</i>	2	0
CP000323.1	<i>Psychrobacter cryohalolentis</i>	1	0
CP000425.1	<i>Lactococcus lactis</i> subsp. <i>cremoris</i>	2	0
CP000539.1	<i>Acidovorax</i> sp.	21	15
DQ017928.1	Uncultured bacterium	3	0
DQ067012.1	Uncultured bacterium	1	0
DQ076434.1	Uncultured <i>Flectobacillus</i> sp.	1	0
DQ117534.1	Bacterium #WM-A4	0	1
DQ165180.1	Uncultured bacterium	0	3
DQ202198.1	Uncultured bacterium	2	0
DQ211400.1	Uncultured bacterium	1	0
DQ211452.2	Uncultured <i>Bacteroidetes/Chorobbi</i> group bacterium	0	2 <sup>b</sup>
DQ221470.1	Uncultured bacterium	0	2
DQ228418.1	Uncultured bacterium	1 <sup>b</sup>	0
DQ256330.1	Uncultured bacterium	1	0
DQ256357.1	Uncultured bacterium	3	1
DQ293994.1	<i>Pleurocapsa</i> sp.	1	0
DQ294626.1	<i>Diaphorobacter</i> sp.	9	3
DQ295866.1	<i>Pelosinus fermentans</i> strain	1	0
DQ316827.1	Uncultured <i>Bacteroidetes</i> bacterium	4	3
DQ336990.1	Uncultured bacterium	3	2
DQ337018.1	Uncultured bacterium	3	6
DQ337585.1	<i>Kaistia</i> sp.	1	0

Appendix 2. Table A1 (continued)

DQ340193.1	Uncultured <i>Mycoplasma</i> sp.	12	4
DQ342824.1	Uncultured bacterium	1	0
DQ354708.1	Uncultured bacterium	0	1
DQ354709.1	Uncultured bacterium	5	5
DQ378249.1	Uncultured soil bacterium	0	1
DQ396035.1	Uncultured organism	1 <sup>b</sup>	0
DQ404678.1	Uncultured bacterium	1	0
DQ409957.1	Uncultured gammaproteobacterium	0	1
DQ413165.1	<i>Sphingobium</i> sp.	2	0
DQ447856.1	Uncultured bacterium	2	0
DQ447857.1	Uncultured bacterium	0	1
DQ456408.1	Uncultured bacterium	4	0
DQ463263.1	Uncultured bacterium	2	0
DQ532127.1	Uncultured bacterium	14	4
DQ532191.1	Uncultured bacterium	0	1
DQ532278.1	Uncultured bacterium	1 <sup>b</sup>	0
DQ538104.1	Uncultured bacterium	0	1
DQ642353.1	Uncultured bacterium	0	1 <sup>b</sup>
DQ675503.1	Uncultured bacterium	1	0
DQ676998.1	Iron-reducing enrichment	1	0
DQ677850.1	Uncultured gammaproteobacterium	2 <sup>b</sup>	0
DQ815250.1	Uncultured bacterium	0	1
DQ824612.1	Uncultured bacterium	1	0
DQ824738.1	Uncultured bacterium	1	0
DQ828466.1	Uncultured actinobacterium	1	0
DQ829086.1	Uncultured proteobacterium	1	0
DQ829228.1	Uncultured <i>Chloroflexi</i> bacterium	1 <sup>b</sup>	0
DQ820310.1	Uncultured actinobacterium	0	1
DQ905990.1	Uncultured <i>Bacteroidetes</i> bacterium	1	1
DQ980905.1	Uncultured bacterium	1	0
DQ990935.1	Uncultured bacterium	1 <sup>b</sup>	0
EF018188.1	Uncultured bacterium	0	1
EF018676.1	Uncultured <i>Bacteroidetes</i> bacterium	0	1
EF029243.1	Uncultured bacterium	1	0
EF029379.1	Uncultured bacterium	2	0
EF029394.1	Uncultured bacterium	3	0
EF029422.1	Uncultured bacterium	1	0
EF029789.2	Uncultured bacterium	1	0
EF032665.1	Uncultured alphaproteobacterium	1	0
EF033499.1	Uncultured <i>Acidovorax</i> sp.	14	10
EF033514.1	<i>Acidovorax</i> sp.	2	4
EF061133.1	<i>Sphingomonas</i> sp.	2	1
EF061965.1	Uncultured gammaproteobacterium	3	1
EF071401.1	Uncultured <i>Firmicutes</i> bacterium	2	0
EF095770.1	<i>Alcaligenes</i> sp.	0	1
EF111185.1	Uncultured betaproteobacterium	0	1
EF196977.1	Uncultured proteobacterium	0	1
EF197017.1	Uncultured proteobacterium	1	0
EF208659.1	Uncultured bacterium	1	0
EF221160.1	Uncultured alphaproteobacterium	1 <sup>b</sup>	0
EF392911.1	Uncultured bacterium	1	0
EF392912.1	Uncultured bacterium	1 <sup>b</sup>	0
EF399436.1	Uncultured bacterium	0	1
EF409261.1	Uncultured bacterium	4	3
EF409289.1	Uncultured bacterium	1	0
EF409294.1	Uncultured bacterium	1	0
EF409299.1	Uncultured bacterium	4	0
EF409306.1	Uncultured bacterium	21	15
EF427922.1	Uncultured bacterium	1	0
EF429742.1	Uncultured bacterium	1	0
EF446186.1	Uncultured bacterium	1	0
EF488749.1	<i>Lysobacter</i> sp.	0	1
EF507945.1	Uncultured bacterium	0	1

Appendix 2. Table A1 (continued)

EF511193.1	Uncultured bacterium	0	1
EF515472.1	Uncultured bacterium	1	0
EF525671.1	<i>Acinetobacter baumannii</i>	2	0
EF540427.1	Uncultured soil bacterium	1	0
EF540468.1	<i>Dietzia</i> sp.	2	0
EF540479.1	<i>Sphingopyxis</i> sp.	1	0
EF554889.1	<i>Ralstonia</i> sp.	1	0
EF555515.1	<i>Geobacillus</i> sp.	1	0
EF574595.1	Uncultured bacterium	3	0
EF574595.1	Uncultured bacterium	7	0
EF590029.1	Uncultured bacterium	0	2
EF590043.1	Uncultured bacterium	2 <sup>b</sup>	0
EF613734.1	Uncultured <i>Clostridiaceae</i> bacterium	0	1
EF632919.1	Uncultured bacterium	0	3
EF660493.1	Uncultured bacterium	1	1
EF670442.1	<i>Propionibacterium acnes</i>	1	0
L14626.1	<i>Arcobacter butzlerii</i>	1	0
X84680.1	<i>Corynebacterium vitrumen</i>	29	0
X95305.1	<i>Acinetobacter</i> sp.	11	4
Y14146.1	<i>Burkholderia</i> sp.	0	1
Z49719.1	<i>Legionella bozemanii</i>	1	0
	Totals	486	163
	<sup>a</sup> E value > 10 <sup>-2</sup>		
	<sup>b</sup> E value < 10 <sup>-3</sup>		
	All other E values = 0		