HUDSON

Bacterial Isolates detected in Devils Lake (not detected in the Sheyenne/Red River Drainage)

Bacterial Sample Collection Methods:

A standard sample size of 60 fish was the objective for each of the target species of fish. A 60 fish sample size provides a 95% confidence level that a pathogen in an infected fish will be detected given in a 5% presumed prevalence of infection in that population (>2000). Fish were captured by used of experimental gill nets, and modified Fyke nets. After capture the fish were transported to a temporary field laboratory at Devil's Lake and held alive with Devil's Lake water until time of necropsy, examination, and sample collection.

Fish were humanely euthanized with tricaine methanesulfonate (MS-222/Finquel). Following an external examine for clinical signs of disease, the body cavity of the fish was aseptically opened by a ventral incision to provide a sterile access to the kidney tissue. A disposable sterile loop (1.0 or 10.0 ul) was inserted into the kidney and the collected sample was streaked onto the surface of Brain-Heart Infusion agar. The test tubes of BHIA were incubated at 22◦C and observed at 24, 48 and 72 hours for bacterial growth. The BHIA tubes were discarded if the culture remained free of bacterial growth after 10 days. Suspect bacterial growth was sub-cultured for isolation of pure cultures. The pure cultures were differentiated by standard biochem profiling and motility testing by hanging drop methodology.
The isolated bacteria were identified using the following commercial assays, API 20E (bioMerieux Vitek, Inc), Biolog Microbial ID/Characterization (Hayward, CA).

2006 Results (Devil's Lake)		**D.L. Species harboring isolate**		
Genus	**Species**		**Detected in Red River Sheyenne Drainage**	**Detected in Canada**
Acintobacter	*lwoffii*	WAE	yes	yes
Aeromonas	*hydrophila*	BLC,FHM,NOP	yes	yes
Brevundimonas	*diminuta*	NOP	no	no
Hafnia	*alvei*	FHM,NOP,WHB	yes	yes
Pseudomonas	*fluorscens*	FHM	yes	yes
Pseudomonas	*mendocina*	FHM	no	no
Yokenella	*regensburgii*	FHM,NOP,WHB	no	no

2007 Results (Devil's Lake)		**D.L. Species harboring isolate**		
Genus	**Species**		**Detected in Red River Sheyenne Drainage**	**Detected in Canada**
Aeromonas	*hydrophila*	BLC, FHM,NOP,WHB, WHS	yes	yes
Hafnia	*alvei*	NOP	yes	yes
Shewanella	*putrefaciens*	WHB	(in Lake Ashtabula in 2008)	no

2008 Results (Devil's Lake)		D.L. Species harboring isolate		
Genus	**Species**		**Detected in Red River Sheyenne Drainage**	**Detected in Canada**
Hafnia	*alvei*	2-NOP	yes	yes
Shewanella	*putrefaciens*	2-YEP	yes (Lake Ashtabula)	no

1> *Pseudomonas mendocina*

1st isolated in soil and water samples in Mendoza, Argentina.

Publications as to pathology in aquatic (or terrestrial) animals not found.
(Search engines used, Google Scholar, Scirus, OJOSE, PubMed)

Multiple publications available on research as to the use of this organism as a bio-remediation agent for toluene and tetrachloro-ethylene (TCE), aromatic hydro-carbons, organo-phospahte compounds and other environmental pollutants

Risk Assessments:

(1) Life History (risk potential)

Life History: aerobic, gram negative motile rod. Found in soils, water.
Use of and/or culture of this organism in a laboratory setting requires only Bio-safety Level one (use of gloves, open bench top use with standard microbial practices). Characterized as agent not known to cause disease in healthy animals.

Since organism is characterized as "not known to cause disease in healthy animals" the risk of infection is "unlikely"-**Rank -1**

(2) Environmental Risk Potential:

Commonly found in soils and waters world-wide.
May have potential for bio-remediation of numerous environmental pollutants.

Since this organism is ubiquitous, is not know to cause disease in healthy animals and may have potential for future use as a bio-remediation agent, the environmental factors that may trigger an outbreak are non-existent. **Rank-1**

(3) Risk of Transfer of this organism:

Since this organism is an ubiquitous soil and water bacteria, it has potential to be isolated world wide, in environmental samples, but publications as to detection in animal tissue were not found, and since it is characterized as not known to cause disease in healthy animals, the risk of transfer is minimal. **Rank-1**

(4) Geographical Distribution of this organism:

Since this organism is an ubiquitous soil and water bacteria, it has potential to be isolated world wide, in environmental samples, but publications as to detection in animal tissue were not found, and since it is characterized as not known to cause disease in healthy animals, the risk of transfer is minimal. **Rank-1**

2> *Yokenella regensburgei*

Isolated in obscure locations in nature, water sources.

Found in the intestinal tracts of insects.

Publications as to pathology in aquatic (or terrestrial) animals not found.
(Search engines used, Google Scholar, Scirus, OJOSE, PubMed)

(1) Life History (risk potential)

Life History: aerobic, gram negative motile rod. Found in intestinal tracts of insects.
Rarely isolated in obscure locations in nature, water sources.
Use of and/or culture of this organism in a laboratory setting requires only
Bio-safety Level one (use of gloves, open bench top use with standard
microbial practices). Characterized as agent not known to cause disease
in healthy animals.

Since organism is characterized as "not known to cause disease in healthy animals" the risk of infection is unlikely and since publications as to pathology in aquatic (or terrestrial) animals not found. **Rank-1**

(2) Environmental Risk Potential:

Since this organism is flora cultured from the intestinal tract of insects, and only rarely isolated in water, and combined with the lack of documentation of aquatic animal pathology, the environmental risk of a disease outbreak is minimal. **Rank-1**

(3) Risk of Transfer of this organism:

There is a lack of documentation on the pathology of this organism in aquatic animals, and also lack of documentation as to the function of this bacterium in the GI-tract of insects (normal flora vs clinical tissue response to this organism in the insect host).
Since this bacteria is characterized as an agent not known to cause disease in healthy animals, the risk of transfer should be considered minimal. **Rank-1**

(4) Geographical Distribution of this organism:

Since this organism is characterized as only being isolated in obscure locations in nature, (a few water samples) the geographical distribution is probably rare in environmental samples.
Without publications as to it's isolation, pathology (if any), occurrence and distribution in insects and/or aquatic animals, the geographical occurrence of this organism in animal tissue cannot be assessed.
Rank-1

3> *Brevundimonas diminuta*

Characterized as "doubtful of any clinical significance

Due to its small size, this bacterium is used as a control organism for validating the efficiency of membranes and filters of water filtering systems used by pharmaceutical and drink water treatment systems.

Also belongs to a group of bacteria-the phosphotriesterases (possessing organophosphate hydrolases) which have been demonstrated to hydrolyze phosphotriesters (used in organo-phospahte insecticides and chemical warfare agents) and is currently being studied for use as a bio-remediation agent for such compounds.

(1) Life History (risk potential)

Life History: aerobic, gram negative motile rod. Common in soil and moist environments.
Use of and/or culture of this organism in a laboratory setting requires only
Bio-safety Level one (use of gloves, open bench top use with standard
microbial practices). Characterized as agent not known to cause disease
in healthy animals.

Since the historical documentation of this organism is listed as doubtful of any clinical significance, it risk as a pathogen is non-existent. **Rank-1**

(2) Environmental Risk Potential:

This organism is actually used in the ASTM Standard Method F838-05 to validate the efficiency of liquid filtration systems, it poses no environmental risk potential. Rank-1

(3) Risk of Transfer of this organism:

Since this organism is listed as doubtful of any clinical significance, its risk of transferring disease is non-existent. **Rank-1**

(4) Geographical Distribution of this organism:

Commonly isolated in soil and moist environments, and commercially distributed for ASTM Method F838-05 (ATTC # 19146). Doubtful clinical significance. **Rank-1**