

Abstract

Bacteria were collected from lesions on six diseased Young of the Year (YOY) Smallmouth Bass from the West Branch Susquehanna River at Watsonstown and cultured on Tryptic Soy or R2A agar. Similar lesions have been observed on smallmouth bass for more than ten years without a known causative agent. The nearly complete 16s rRNA gene was amplified from isolates, and the 5' end was sequenced via conventional Sanger methods. Identifications were made by comparison of the quality-trimmed sequence to the EZTaxon type strain database. The most commonly isolated organisms were *Plesiomonas shigelloides*, *Chryseobacterium gambrini*, and a variety of *Aeromonas* species, including *A. australiensis*, *A. veronii*, and *A. taiwanensis*. Other less frequently isolated organisms were *Acinetobacter gyllenbergii*, *Flavobacterium johnsonii*, *Exiguobacterium acetylicum*, *Pseudomonas mosselii*, and a novel *Chryseobacterium* species. Additional testing must be conducted to determine whether any of these organisms is the causative agent for Smallmouth Bass disease.



Figure 1: Lesions on two of the six sampled YOY Smallmouth Bass provided by Geoffrey Smith, PA Fish and Boat Commission

Methods



Figure 2: Initial streaks from swabbed Smallmouth Bass lesions

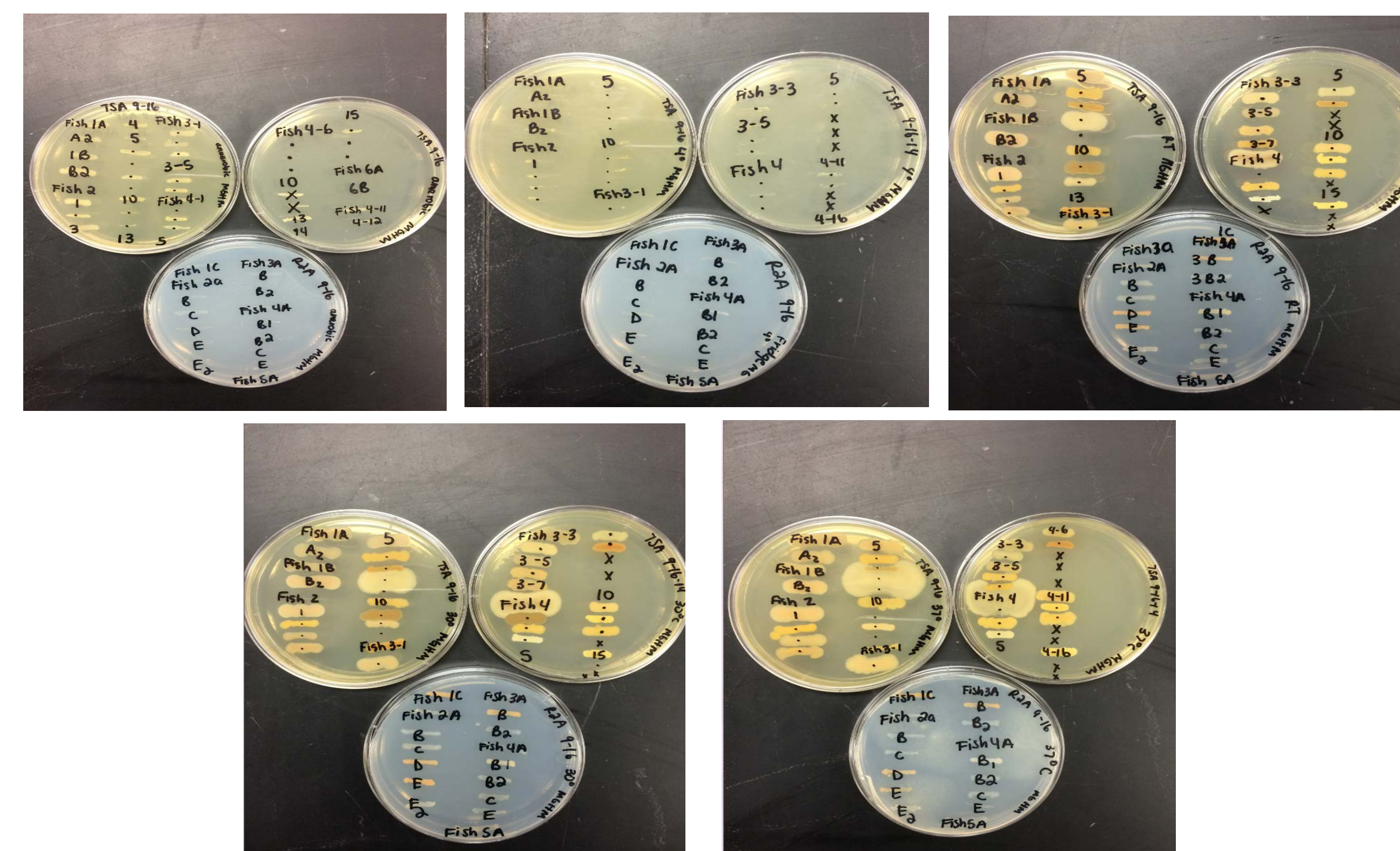


Figure 3: Growth analyzed under five separate conditions (anaerobic, 4°C, Room Temperature, 30°C, and 37°C)

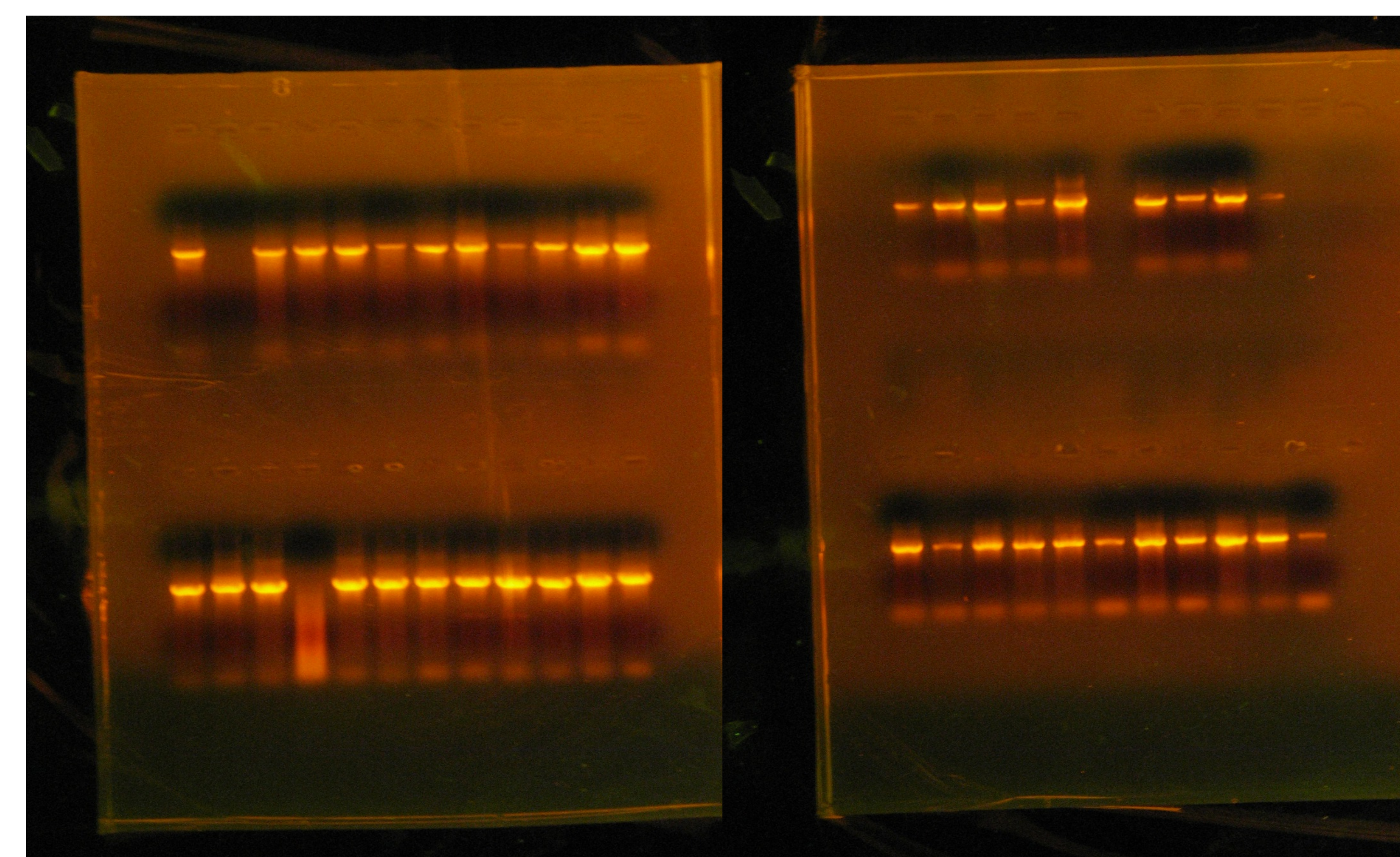


Figure 4: gel electrophoresis of prepared PCR samples

Results

Genus Species	strain	16S rRNA %ID
<i>Aeromonas australiensis</i>	SMB1A	99.85
<i>Aeromonas australiensis</i>	SMB 1A2	100
<i>Serratia fonticola</i>	SMB1B	98.06
<i>Citrobacter freundii</i>	SMB2C	98.72
<i>Exiguobacterium indicum</i>	SMB2D	99.63
<i>Serratia fonticola</i>	SMB2E	94.45
<i>Acinetobacter gyllenbergii</i>	SMB2E2	98.59
<i>Aeromonas hydrophila</i> complex	SMB 2-1	100
<i>Aeromonas australiensis</i>	SMB2-3	100
<i>Exiguobacterium acetylicum</i>	SMB 2-4	98.86
<i>Pseudomonas mosselii</i>	SMB 2-5	99.46
<i>Exiguobacterium acetylicum</i>	SMB2-6	99.6
<i>Chryseobacterium gambrini</i>	SMB 2-7	99.58
<i>Plesiomonas shigelloides</i>	SMB 2-8	100
<i>Chryseobacterium gambrini</i>	SMB2-9	98.49
<i>Exiguobacterium acetylicum</i>	SMB 2-10	98.87
<i>Flavobacterium johnsoniae</i>	SMB 2-11	99.48
<i>Rhizobium neoptum</i>	SMB2-12	99.04
<i>Vogesella perlucida</i>	SMB2-13	99.03
<i>Shewanella oneidensis</i>	SMB2-14	99.74
<i>Aeromonas veronii</i> complex	SMB3A	100
<i>Exiguobacterium acetylicum</i>	SMB3B	99.73
<i>Chryseobacterium lactis</i>	SMB 3-1	99.08
<i>Aeromonas australiensis</i>	SMB3-3	99.59
<i>Aeromonas veronii</i> complex	SMB3-5	99.41
<i>Aeromonas veronii</i> complex	SMB3-6	100
<i>Aeromonas veronii</i> complex	SMB3-7	95.51
<i>Aeromonas australiensis</i>	SMB4A	100
<i>Pseudomonas hunanensis</i>	SMB4B	99.74
<i>Pseudomonas mosselii</i> complex	SMB4B2	99.88
<i>Bacillus toyonensis</i> complex	SMB4-1	100
<i>Flavobacterium johnsoniae</i>	SMB4-2	99.6
<i>Exiguobacterium indicum</i>	SMB4-3	90.45
<i>Staphylococcus epidermidis</i>	SMB4-4	87.93
<i>Chryseobacterium gambrini</i>	SMB4-5	99.85
<i>Acinetobacter gyllenbergii</i> CIP	SMB4-6	99.21
<i>Candidatus Chryseobacterium massiliae</i>	SMB4-7	97.75
<i>Plesiomonas shigelloides</i>	SMB4-8	100
<i>Candidatus Chryseobacterium massiliae</i>	SMB 4-9	97.66
<i>Aeromonas veronii</i> complex	SMB4-10	96.76
<i>Acinetobacter junii</i>	SMB4-11	97.35
<i>Micrococcus luteus</i> complex	SMB4-12	99.6
<i>Exiguobacterium acetylicum</i>	SMB4-13	100
<i>Chryseobacterium gambrini</i>	SMB4-14	99.61
<i>Plesiomonas shigelloides</i>	SMB4-15	99.52
<i>Micrococcus luteus</i> complex	SMB4-16	99.86
<i>Plesiomonas shigelloides</i>	SMB4-17	100
<i>Chromobacterium haemolyticum</i>	SMB4-18	99.73
<i>Aeromonas taiwanensis</i>	SMB5a	100
<i>Aeromonas hydrophila</i> complex	SMB 6A	99.48
<i>Aeromonas hydrophila</i> complex	SMB 6B	99.73

Table 1: Strain identifications based on 16S rRNA sequence analysis.

Conclusions

- *Aeromonas australiensis*: present in four of six sampled bass - a known human and fish pathogen.
- *Exiguobacterium acetylicum*: present in three of six bass
- *Serratia fonticola*: present in two bass
- *Aeromonas hydrophila*: present in two bass- a known fish pathogen
- *Pseudomonas mosselii*: present in two bass- a known human and fish pathogen
- *Chryseobacterium gambrini*: present in two bass- known human and fish pathogen
- *Plesiomonas shigelloides*: present in two bass- a known human and fish pathogen
- *Flavobacterium johnsoniae*: present in two bass- a known fish pathogen
- *Exiguobacterium indicum*: present in two bass
- *Acinetobacter gyllenbergii*: present in two bass-
- *Aeromonas veronii*: present in two bass – a known fish pathogen.
- ***Candidatus Chryseobacterium massiliae* probable novel species**

Future Work

Future work will include testing pathogenicity of isolated and identified species. Species that were present in multiple fish will be used to re-infect healthy YOY Smallmouth Bass to determine which species are the likely disease causing agents

References

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