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Identification of *Bacteroides* Species in Human and Fish by Culture and Molecular Techniques

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Motivation

- Identifying sources of fecal contamination in environmental samples is critical to develop effective pollution remediation strategies.
- *Bacteroides*-specific molecular markers have been widely used to discriminate human sources of fecal contamination from other sources in environmental samples. Our recent work showed significant cross amplification of several published assays for quantification of human-specific *Bacteroides* 16S rRNA molecular markers with fecal DNA from fish species: tilapia, catfish, trout and salmon (McLain et al, 2009).
- We extended this work using culturing of *Bacteroides* isolates from human and fish fecal samples.

Objectives

To culture *Bacteroides* isolates from human and fish fecal samples and identifying the species using API tests for:

- Validating our molecular techniques
- Comparing species using PCR result
- Determining most prevalent species in human versus fish feces

Results

Table 1. *Bacteroides* species in human and fish samples identified by culture and molecular techniques

Samples	Identification Technique		
	Bacteroides Bile Esculin Agar (BBE)	API Strips (Rapid ID 32A)	Sequences: % Match of Identified *Bacteroides* species using API strips with sequences from NCBI
Human	Brown to black colonies surrounded by a brown zone in the medium	Excellent Identification to *B.volgatus* (%ID = 99.9, T=0.75)	(534bp) 99% match to Cultured *B.volgatus* (AB510712)
Nile tilapia	Brown to black colonies surrounded by a brown zone in the medium	Good Identificationto *B.eggerthii* (%ID = 99.8, T = 0.41)	(675bp) 98% match to uncultured *Bacteroides* of Yellow Catfish (Pelteobagrus fulvidraco) (GQ36002)
Grass carp	Brown to black colonies surrounded by a brown zone in the medium	Good Identification to the Genus, *B. uniformis*(%ID = 59, T=0.5) *B. ovatus*(%ID = 32.3,T =0.51) *B.stercoris* (%ID = 5.7, T = 0.34)	(675bp) 97% match to uncultured *Bacteroides* from Gull feces (FJ220880)
Blue catfish	Brown to black colonies surrounded by a brown zone in the medium	Good Identification to the Genus, *B. uniformis*(%ID = 59, T=0.5) *B. ovatus*(%ID = 32.3,T =0.51) *B.stercoris* (%ID = 5.7, T = 0.34)	(675bp) 97% match to uncultured *Bacteroides* from Gull feces (FJ220880)
Channel catfish	Brown to black colonies surrounded by a brown zone in the medium	Good Identification to the Genus, *B. uniformis*(%ID = 59, T=0.5) *B. ovatus*(%ID = 32.3,T =0.51) *B.stercoris* (%ID = 5.7, T = 0.34)	(675bp) 97% match to uncultured *Bacteroides* from Gull feces (FJ220880)

Excellent Identification
%ID >= 99.9 &
T > = 0.75
Very Good Identification
%ID >= 99.0 &
T > = 0.5
Good Identification
%ID >= 90.0 &
T > = 0.25
Acceptable Identification
%ID >= 80.0 &
T > = 0

T: Reliability of identification test result
NCBI: National Center for Biotechnology Information (NCBI) GenBank data base

Summary

- *Bacteroides* species from human and tilapia were identified.
  Human → *B. volgatus*
  Tilapia → *B. eggerthii*
- *Bacteroides* isolates from grass carp, channel catfish and blue catfish feces were matched with *B. uniformis*, *B. ovatus* and *B. stercoris* using Rapid ID 32A strip. This is the first report of these *Bacteroides* isolates at the acceptable level in the above fish species. The sequencing results of these isolates matched most closely with uncultured *Bacteroides* from Gull feces (Table 1).
- *Bacteroides* cultured from human feces were identified using the Rapid ID 32A strip and by the sequencing results. However, the culture and sequencing results of cultured fish samples (tilapia, grass carp, channel catfish and blue catfish) were identified but matched only with the sequences of uncultured isolates listed in the NCBI GenBank.
- These reports of sequences from fish feces highlights the uncultured diversity of *Bacteroides* strains that may exist in fish species and water fowl including Gull.

Experimental Approach

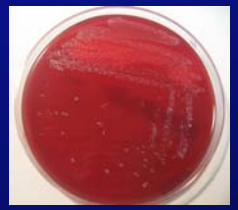

Nile tilapia

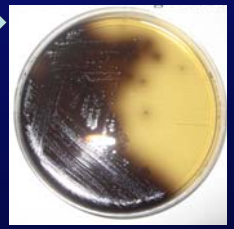

Channel catfish


Blue catfish


Grass carp

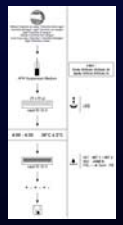
Fecal Sample Collected


Blood Agar


Bacteroides Bile Esculin Agar

Culturing *Bacteroides*


Anaerobic bag and Chopped meat medium



API Strips (Rapid ID 32A Strips)

PCR and DNA Sequencing

Further Study

- Additional fish species widely used for stocking ponds in the United States, including trout, will be studied utilizing these techniques.
- Clone libraries of full-length *Bacteroides*16S rRNA genes of fish species will be constructed.
- More specific source tracking primers and probes for Human-specific *Bacteroides* that consider the potential for fish fecal contamination in water samples, will be developed for qPCR.

Literature Cited

McLain J., H. Ryu, L. Kabiri, C. Rock & M. Abbaszadegan. 2009. Lack of specificity for PCR assays targeting human Bacteroides 16S rRNA gene: cross-amplification with fish feces. *FEMS Microbial Lett* **299:**38-43.

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