# Characterization of the *Flavobacteriaceae* using Multilocus Sequence Typing

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## Abstract:

This work is a continuation of research done under the ASM undergraduate research fellowship. Four genes: glutamine sythetase, DNA gyrase B, chaperonin GroEL, and DNA directed RNA polymerase subunit B, were studied using multilocus sequence typing to characterize the phylogenetic relationships between members of the *Flavobacteriaceae*. Degenerate oligonucleotide primers were created for the four genes, and the resulting gene fragments were then sequenced by Agencourt. A multiple sequence alignment was performed on the data, phylogenetic trees and difference tables were also generated.


## The Problem:

With the newest revision to the characterizations required to publish novel species, 16S rRNA is no longer sufficient, and other means must also be utilized. MLST can be used to supplement the 16S data for publication.

## Method:

- *Chryseobacterium gleum* and similar sequences were used to find highly conserved regions of the genes
- Degenerate oligonucleotide primers were synthesized
- PCR was run to amplify the selected gene fragments, which were then sequenced
- Contiguous sequences were compiled using CAP3, and the contigs were aligned using the ClustalW algorithm in MEGA4
- Weighted neighbor joining phylogenetic trees and difference tables were generated

## Goals:

To clarify and improve the phylogenetic relationships between members of the *Flavobacteriaceae.*

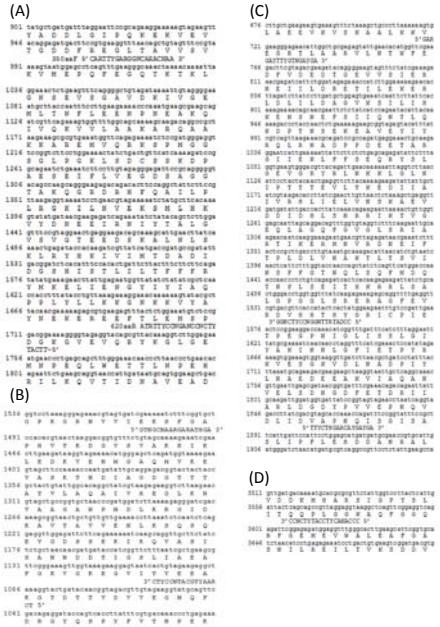


Figure 1: Depiction of the open reading frames and primer binding sites for (A) gyrB, (B) groEL, and (C)/(D) rpoB.

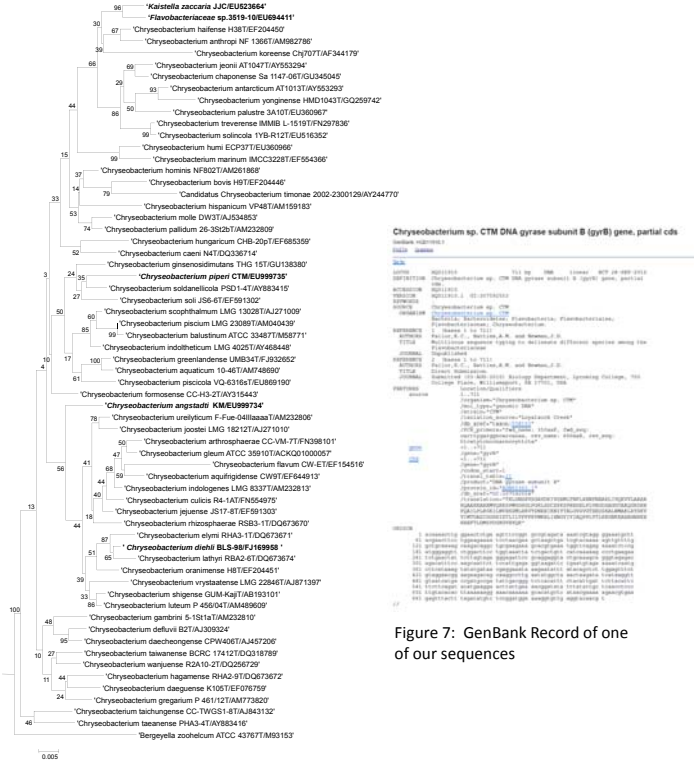


Figure 2: Phylogenetic relationship of the 16S rRNA gene. Generated using the Weighted Neighbor Joining algorithm and data from the Ribosomal Database Project.




Figure 7: GenBank Record of one of our sequences

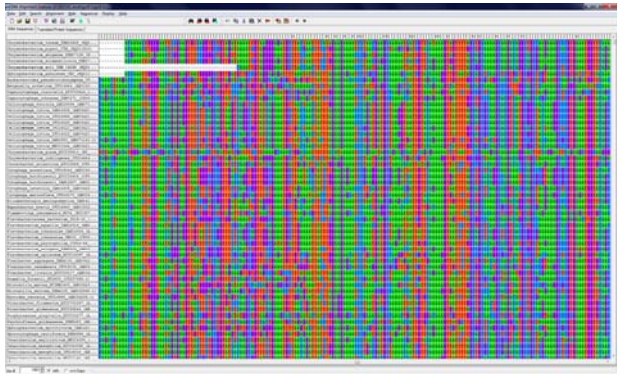

Figure 8: Multiple Sequence alignment using the ClustalW algorithm in MEGA4

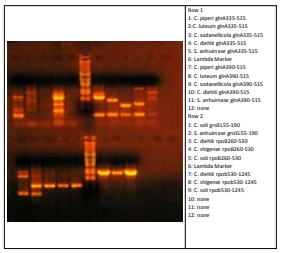

Figure 3: Gel used to estimate DNA concentrations before sending to Agencourt for sequencing.

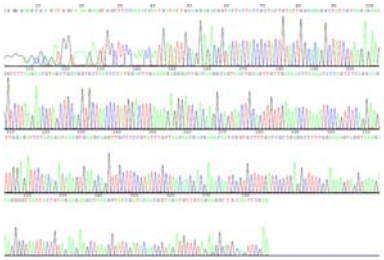

Figure 4: Typical electropherogram of DNA sequence.

Figure 5: CAP3 assembly of complementary strands into a single contiguous sequence.

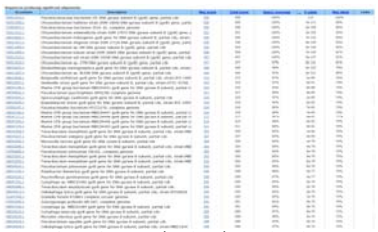

Figure 6: BLASTn search using the strain JJC DNA gyrase subunit B gene fragment.

Figure 9:Phylogenetic relationships of *Flavobacteriaceae* as shown by phylogenetic tree and difference tables. (A) gyrB, (B) groEL, (C) rpoB.

## Conclusions:

- percent identity is found between members of the same genus
- percent identity is found between the different genera
- Strain JJC, *Flavobacteriaceae bacterium* 3519-10 and *Chryseobacterium haifense* are sufficiently different enough to be in a different genus.

## Refrences:


**Huang, X. & Madan, A. (1999**) CAP3: A 285 DNA sequence assembly program. *Genome Res.* 9, 868-877

**Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007).** MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24:1596-1599**.**

**Tindall, B., Rossello-Mora, R., Busse, H., Ludwig, W., Kampfer, P. (2010)** Notes on the characterization of prokaryote strais for taxonomic purposes. *Int J Syst Evol Microbiol.* 60:249-266.

**See also:** Poster by Jordan Krebs