



Molecular and Phenotypic Characteristics of a Novel Species and Related Type Strains Suggest a Reorganization within the Genus *Chryseobacterium*

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Abstract:

In the field of Microbial Taxonomy and Systematics, there are lumpers and splitters. Lumpers tend to group species together into fewer, more diverse groups, while splitters tend to create new, more homogeneous groups. The genus *Chryseobacterium* has increased from 5 to 58 species in the past decade, due to the description of new species, and movement of other species into the genus. *Chryseobacterium koreense* was initially named *Kaistella koreensis* (Kim et al. 2004) then was reclassified when *Chryseobacterium anthropi*, sp. was published (Kampfer et al. 2009) and placed into the *Chryseobacterium* genus based on its similarity to *Chryseobacterium halifense*.

Our studies with strain JJC, a novel species candidate, closely related species, and species that are more distantly related but still within the genus *Chryseobacterium* have identified several phenotypes, that clearly distinguish these organisms into two groups. We recommend reinstatement of the genus *Kaistella* for organisms within the larger *Chryseobacterium* group that contain a high percentage of the fatty acid 15:0 anteiso, do not produce flexirubin pigments,...

Introduction/Background:

1. *Chryseobacterium* sp. JJC was isolated from the Loyalsock Creek and was initially characterized as an "unknown" in Bio 321W – Microbiology

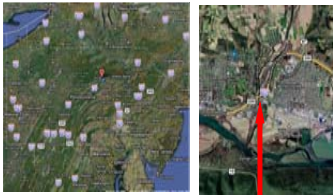


Figure 1. Collection Site: Loyalsock Creek

Methods:

- Environmental unknowns cultured & characterized in Microbiology course
- Colony PCR of 16S rDNA with primers 27f & 1492r, 1 Sanger sequencing rxn
- Compare sequence to validly published type strains (Eztaxon.org)

- Fully sequence both strands of nearly complete 16S rDNA
 - Submit Sequence to GenBank
 - ClustalW Alignment, Neighbor Joining Tree to infer phylogenetic relationships
- Morphological/Metabolic characterization w/standard tests
 - Colony morphology, color, Gram stain, wet mount
 - Temperature, O₂, pH, NaCl requirements
 - Carbohydrate & Nitrogen metabolism
 - Exoenzymes, differential and selective medium
- API Test Strips (50 CH, 20E/NE, ZYM)
- Fatty Acid Methyl Ester (FAME) Analysis (MIDI)
- Biolog GenIII metabolic profile
- Family-specific tests such as Multi Locus Sequence Analysis, pigment analysis, respiratory quinones, polar lipids, cell wall amino acids.

- Deposit Strains in Culture Collections (e.g. ATCC, DSMZ, CCGU, JCM, ARS/NRRL)

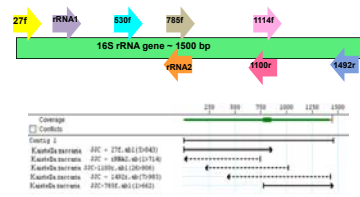


Figure 2. 16S rDNA Sequence Analysis – Top schematic shows the location of PCR and sequencing primers. Lower diagram illustrates the reads assembled into the final consensus sequence.

Figure 3. Best Type Strain matches to JJC using EzTaxon.org.



Figure 4. Weighted Neighbor joined tree generated for 16S rDNA sequence analysis.

The evolutionary history was inferred using the Neighbor-Joining method (Caitou and Nei, 1987). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007). The blue star indicates the group to be reclassified back into the Kaistella genus.

Table 1. Fatty Acid Methyl Ester (FAME) analysis. Numbers correspond to percent of fatty acid from all fatty acids. Data for *Chryseobacterium solincola*, *C. antarcticum*, *C. jeonii*, *C. palustre*, *C. treverense*, *C. anthropi*, *C. chaponense*, and *C. koreense* not from this study, sources as indicated in chart.

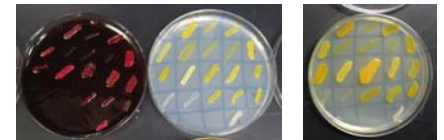


Figure 5. Growth of *Chryseobacterium* strains on Endo, R2A and Cetrime agars.

Conclusions:

- Strain JJC will be named as *Kaistella zaccaria*, sp. This organism represents a group of *Chryseobacterium* that should be reclassified back into the *Kaistella* genus.
- Reclassification of a group of *Chryseobacterium* into the genus *Kaistella* is supported by these organisms having a higher 15:0 anteiso than other *Chryseobacterium* members.
- To complete the preparation of these strains for publication in the *International Journal of Systematic and Evolutionary Microbiology*, several additional reference strains must be obtained for comparison, including: *Chryseobacterium anthropi* and *Chryseobacterium koreense*.
- For Multi Locus Sequence Analysis results, see "Development of Multi-Locus Sequence Typing for the *Flavobacteriaceae*" by K.C. Failor and Jeffrey Newman, PhD.

Acknowledgments

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