

Genomic and Phenotypic Characterization of Two Freshwater Isolates of a Novel Flavobacterium



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Abstract:

Two Gram-negative, rod shaped, yellow pigmented aerobic bacterial strains, designated JRM and KMS, were isolated from the Loyalsock Creek in Montoursville PA as part of an undergraduate microbiology course. Growth was observed at temperatures 4-30 °C and an optimal pH of 6-9. Comparative 16SrRNA sequences identified the closest match to *Flavobacterium hibernum* and *Flavobacterium hydatis*. Full genome sequencing of both isolates and the reference Type strains was completed and an estimated DNA-DNA Hybridization (eDDH) was calculated among them using the Genome-to-Genome-Distance Calculator. Strains JRM and KMS had an eDDH value of 74.6%, above the 70% for inclusion in the same species. When compared to JRM and KMS, *Flavobacterium hibernum* and *Flavobacterium hydatis* had GGDC values of under 70%. ANI was completed with the full genomes of JRM and KMS versus *Flavobacterium hibernum* and *Flavobacterium hydatis* with the results being under the threshold value for the same species. On the basis of phenotypic and genotypic data, strain JRM and KMS represent a novel species of the genus *Flavobacterium* with the proposed name *Flavobacterium falloni*.

Background:

- Unknown Microbe Lab identifies isolates from a freshwater creek using Biolog GenIII and 16SrRNA.
- Closest relatives *Flavobacterium hydatis* and *Flavobacterium hibernum* obtained for
 - Whole Genome Sequence Analysis
 - Phylogenomic Metrics
 - Genotype-Phenotype Correlation
 - Polyphasic Characterization

Genomic Comparisons

	<i>Flavobacterium falloni</i> sp. JRM	<i>Flavobacterium falloni</i> sp. KMS	<i>Flavobacterium hydatis</i>	<i>Flavobacterium hibernum</i>
Total Reads	826,175	725,291	1,489,773	2,865,011
Contigs	170	58	99	29
Average Coverage	27X	28X	54X	109X
Assembly Length	5,386,118 bp	5,620,217 bp	5,877,671 bp	5,283,662 bp
Accession	JSY001	JSPY01	JRH01	JPRK01

	<i>Flavobacterium JRM</i>	<i>Flavobacterium KMS</i>	<i>Flavobacterium hydatis</i>	<i>Flavobacterium hibernum</i>
<i>Flavobacterium sp. JRM</i>	-	74.2	41.7	23.4
<i>Flavobacterium sp. KMS</i>	96.95	-	40.8	23.3
<i>Flavobacterium hydatis</i>	90.54	90.22	-	23.3
<i>Flavobacterium hibernum</i>	78.36	78.25	78.38	-

Phenotypic Analysis:

	10:0	10:1	10:2	10:3	10:4	10:5
<i>Flavobacterium JRM</i>	1.0	0.0	1.6	1.5	1.2	1.2
<i>Flavobacterium KMS</i>	0.6	0.5	1.6	0.4	1.0	1.0
<i>Flavobacterium hydatis</i>	2.6	2.5	2.0	2.4	2.4	2.4
<i>Flavobacterium hibernum</i>	1.1	0.7	0.5	0.0	0.0	0.0
<i>Flavobacterium falloni</i> sp. JRM	2.1	2.3	1.9	1.5	1.5	1.5
<i>Flavobacterium falloni</i> sp. KMS	1.1	0.5	0.5	0.2	0.2	0.2
<i>Flavobacterium hydatis</i>	1.8	1.5	0.5	0.0	0.0	0.0
<i>Flavobacterium hibernum</i>	2.2	2.6	0.7	1.4	1.4	1.4
16:1 n-7 MeVLC: n-6	12.8	12.9	12.9	12.8	12.8	12.8
<i>C19</i>	2.0	2.0	0.2	0.3	0.3	0.3
<i>MeVLC: n-6</i>	7.0	7.7	7.1	6.1	6.1	6.1
16:0-Dimethyl S1: n-6	6.6	6.4	4.1	0.0	0.0	0.0
<i>Flavobacterium JRM</i>	0.8	0.8	0.4	1.0	1.0	1.0
<i>Flavobacterium KMS</i>	1.4	1.8	1.7	1.2	1.2	1.2
<i>Flavobacterium hydatis</i>	4.7	5.0	0.8	4.5	4.5	4.5
<i>Flavobacterium hibernum</i>	1.7	1.5	1.1	1.7	1.7	1.7
<i>Flavobacterium falloni</i> sp. JRM	1.5	1.8	1.1	1.7	1.7	1.7
<i>Flavobacterium falloni</i> sp. KMS	7.3	6.5	6.2	7.7	7.7	7.7

Antibiotics	<i>F. sp. JRM</i>	<i>F. sp. KMS</i>	<i>F. hydatis</i>	<i>F. hibernum</i>
Kanamycin	S	S	S	S
Erythromycin	S	S	S	S
Penicillin	S	S	S	S
Nalidixic acid	S	S	S	S
Sulamycin	S	S	S	S
Tetracycline	S	S	S	S
Amoxicillin	S	S	S	S
Colistin	S	S	S	S
Amphotericin	S	S	S	S
Chloramphenicol	S	S	S	S
Chloramphenicol	S	S	S	S
Clotrimazole	S	S	S	S

Table 5: HPLC Pigment Comparison

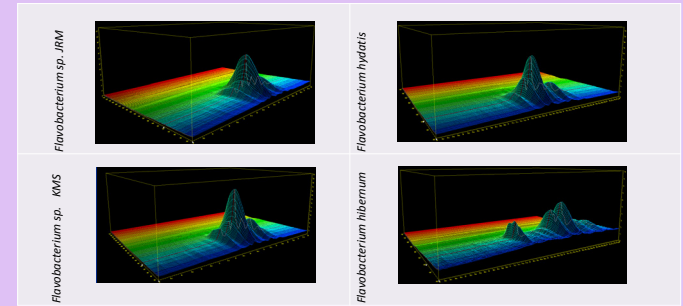


Table 6. BIOLOG GENIII plate results

	JRM	KMS	hydatis	hibernum
001	0.0	0.0	0.0	0.0
002	0.0	0.0	0.0	0.0
003	0.0	0.0	0.0	0.0
004	0.0	0.0	0.0	0.0
005	0.0	0.0	0.0	0.0
006	0.0	0.0	0.0	0.0
007	0.0	0.0	0.0	0.0
008	0.0	0.0	0.0	0.0
009	0.0	0.0	0.0	0.0
010	0.0	0.0	0.0	0.0
011	0.0	0.0	0.0	0.0
012	0.0	0.0	0.0	0.0
013	0.0	0.0	0.0	0.0
014	0.0	0.0	0.0	0.0
015	0.0	0.0	0.0	0.0
016	0.0	0.0	0.0	0.0
017	0.0	0.0	0.0	0.0
018	0.0	0.0	0.0	0.0
019	0.0	0.0	0.0	0.0
020	0.0	0.0	0.0	0.0
021	0.0	0.0	0.0	0.0
022	0.0	0.0	0.0	0.0
023	0.0	0.0	0.0	0.0
024	0.0	0.0	0.0	0.0
025	0.0	0.0	0.0	0.0
026	0.0	0.0	0.0	0.0
027	0.0	0.0	0.0	0.0
028	0.0	0.0	0.0	0.0
029	0.0	0.0	0.0	0.0
030	0.0	0.0	0.0	0.0
031	0.0	0.0	0.0	0.0
032	0.0	0.0	0.0	0.0
033	0.0	0.0	0.0	0.0
034	0.0	0.0	0.0	0.0
035	0.0	0.0	0.0	0.0
036	0.0	0.0	0.0	0.0
037	0.0	0.0	0.0	0.0
038	0.0	0.0	0.0	0.0
039	0.0	0.0	0.0	0.0
040	0.0	0.0	0.0	0.0
041	0.0	0.0	0.0	0.0
042	0.0	0.0	0.0	0.0
043	0.0	0.0	0.0	0.0
044	0.0	0.0	0.0	0.0
045	0.0	0.0	0.0	0.0
046	0.0	0.0	0.0	0.0
047	0.0	0.0	0.0	0.0
048	0.0	0.0	0.0	0.0
049	0.0	0.0	0.0	0.0
050	0.0	0.0	0.0	0.0
051	0.0	0.0	0.0	0.0
052	0.0	0.0	0.0	0.0
053	0.0	0.0	0.0	0.0
054	0.0	0.0	0.0	0.0
055	0.0	0.0	0.0	0.0
056	0.0	0.0	0.0	0.0
057	0.0	0.0	0.0	0.0
058	0.0	0.0	0.0	0.0
059	0.0	0.0	0.0	0.0
060	0.0	0.0	0.0	0.0
061	0.0	0.0	0.0	0.0
062	0.0	0.0	0.0	0.0
063	0.0	0.0	0.0	0.0
064	0.0	0.0	0.0	0.0
065	0.0	0.0	0.0	0.0
066	0.0	0.0	0.0	0.0
067	0.0	0.0	0.0	0.0
068	0.0	0.0	0.0	0.0
069	0.0	0.0	0.0	0.0
070	0.0	0.0	0.0	0.0
071	0.0	0.0	0.0	0.0
072	0.0	0.0	0.0	0.0
073	0.0	0.0	0.0	0.0
074	0.0	0.0	0.0	0.0
075	0.0	0.0	0.0	0.0
076	0.0	0.0	0.0	0.0
077	0.0	0.0	0.0	0.0
078	0.0	0.0	0.0	0.0
079	0.0	0.0	0.0	0.0
080	0.0	0.0	0.0	0.0
081	0.0	0.0	0.0	0.0
082	0.0	0.0	0.0	0.0
083	0.0	0.0	0.0	0.0
084	0.0	0.0	0.0	0.0
085	0.0	0.0	0.0	0.0
086	0.0	0.0	0.0	0.0
087	0.0	0.0	0.0	0.0
088	0.0	0.0	0.0	0.0
089	0.0	0.0	0.0	0.0
090	0.0	0.0	0.0	0.0
091	0.0	0.0	0.0	0.0
092	0.0	0.0	0.0	0.0
093	0.0	0.0	0.0	0.0
094	0.0	0.0	0.0	0.0
095	0.0	0.0	0.0	0.0
096	0.0	0.0	0.0	0.0
097	0.0	0.0	0.0	0.0
098	0.0	0.0	0.0	0.0
099	0.0	0.0	0.0	0.0
100	0.0	0.0	0.0	0.0

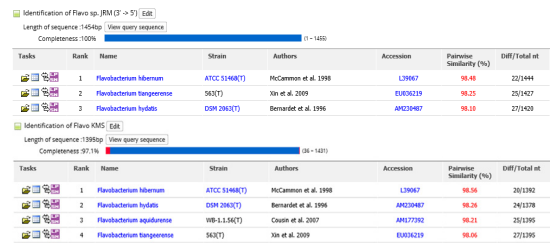


Figure 1. EZ-Taxon 16S rRNA pairwise similarity for JRM and KMS (Kim et al 2012)

Figure 2. Neighbor Joining Tree of nearly full length 16S rRNA sequences.

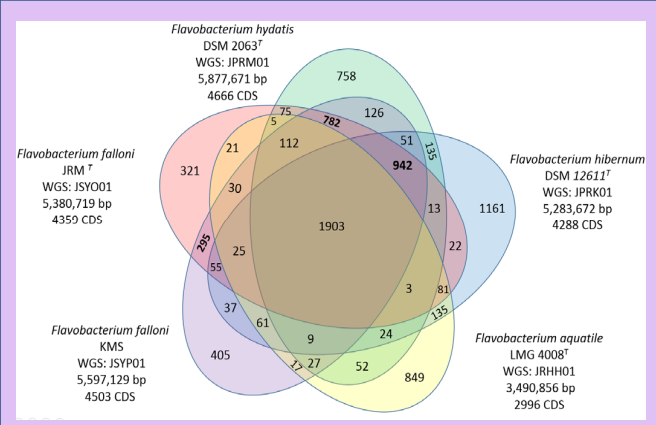
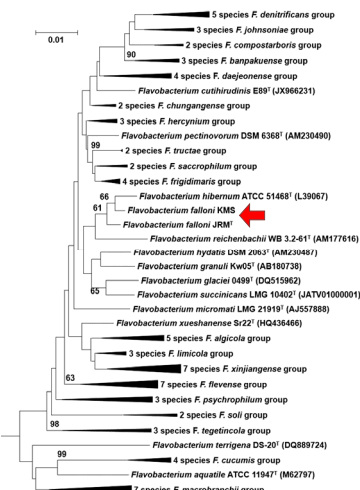


Figure 3. Venn Diagram comparing unique and shared genes between *Flavobacterium falloni* and reference species

Conclusions:

- Estimated DDH and ANI values between JRM and KMS suggest they are the same species as the values lie above the species threshold
- Estimated DDH and ANI values between *F. falloni* and the reference organisms are below the species threshold indicating a new species.
- Flavobacterium aquatile* does not share 942 genes with the other organisms, suggesting a restructuring of the genus *Flavobacterium*.

Conclusions:

- Significant Phenotypic differences observed in the antibiotic sensitivity and in carotenoid production but not in Fatty Acid Composition.
- Flavobacterium sp. JRM* and *Flavobacterium sp. KMS* are both phenotypically and genetically different enough from reference organisms to be considered a novel species

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