CHARACTERIZATION OF *EPILITHONIMONAS* SP. FH1. FROM A FRESHWATER CREEK

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Abstract

Epilithonimonas sp. FH1 is a gram-negative bacterium that was isolated from a freshwater creek and is hypothesized to be a novel species of the genus *Epilithonimonas* within the family *Flavobacteriaceae*. Experimental data was collected on strain FH1 in parallel with the type strains of the only two other species of the genus, *Epilithonimonas lactis* and *Epilithonimonas tenax*. Tests done include multilocus sequence analysis (MLSA), fatty acid methyl ester (FAME) analysis, Biolog GenIII plates, API tests, Kirby-Bauer antibiotic sensitivity testing, NaCl and temperature requirements, exoenzyme presence, and growth capabilities. The 16S rRNA and protein-coding *rpoB*, *groEL*, and *gyrB* were amplified using the polymerase chain reaction and sent to Beckman Coulter Genomics for sequencing. Contigs were assembled in CAP3 contig assembly program and analyzed using EzTaxon and BLAST. Pairwise distance tables and phylogenetic trees were constructed in MEGA5 using sequences from other organisms of the family *Flavobacteriaceae*., FH1 sequences showed a high similarity to *E. lactis* sequences for the protein-coding genes, with the pairwise distance being less than 5% for *rpoB*, *groEL*, and *gyrB*. Therefore, *Epilithonimonas* sp. FH1, despite showing unique phenotypic characteristics, cannot be concluded to be a novel species without supporting DNA-DNA hybridization or complete genome sequence data.

Background

- *Epilithonimonas sp.* FH-1 isolated during water quality testing, displayed flexirubin pigments.
- Only two species of this genus have been described in the literature, each with one strain

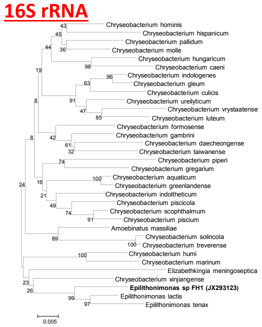
Hypothesis: *Epilithonimonas sp.* FH-1 is a novel species of the genus *Epilithonimonas* and the family *Flavobacteriaceae.*

Methods

- 16S rRNA, rpoB, groEL, and gyrB sequences were amplified using PCR, sequenced, assembled with CAP3, deposited in GenBank.
- Sequences, MEGA5 used to align sequences, construct phylogenetic trees and calculate pairwise distance charts .
- Fatty acid methyl ester (FAME) analysis using GC.
- Carbon source utilization , resistance to inhibitory conditions examined with Biolog GenIII plates.
- Traditional growth and biochemical phenotypes tested
- Antibiotic sensitivity tested using the Kirby-Bauer method.

Results – MLSA

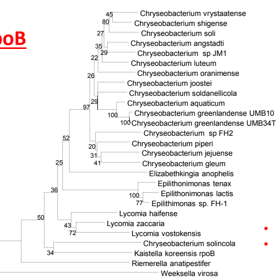
16S rRNA



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1 Epilithonimonas sp FH1 [illegible]																								
2 Epilithonimonas lactis [illegible]	0.021																							
3 Epilithonimonas tenax [illegible]	0.031	0.020																						
4 Chryseobacterium xinjiangense [illegible]	0.043	0.041	0.040																					
5 Chryseobacterium humi [illegible]	0.043	0.044	0.047	0.047																				
6 Chryseobacterium hungaricum [illegible]	0.043	0.050	0.060	0.052	0.049																			
7 Chryseobacterium formosense [illegible]	0.044	0.042	0.045	0.045	0.036	0.042																		
8 Chryseobacterium hominis [illegible]	0.045	0.035	0.044	0.044	0.040	0.035	0.029																	
9 Chryseobacterium indoltheticum [illegible]	0.045	0.052	0.060	0.040	0.045	0.036	0.029	0.008																
10 Chryseobacterium gambrini [illegible]	0.045	0.047	0.049	0.039	0.045	0.044	0.027	0.036	0.036															
11 Chryseobacterium piscicola [illegible]	0.047	0.035	0.041	0.032	0.051	0.050	0.038	0.034	0.028	0.045														
12 Chryseobacterium hispanicum [illegible]	0.048	0.056	0.060	0.052	0.050	0.041	0.042	0.027	0.038	0.042	0.048													
13 Chryseobacterium indologenes [illegible]	0.049	0.050	0.059	0.056	0.049	0.043	0.042	0.034	0.043	0.038	0.045	0.045												
14 Chryseobacterium piscium [illegible]	0.049	0.044	0.050	0.038	0.049	0.049	0.033	0.032	0.025	0.048	0.019	0.044	0.043											
15 Chryseobacterium aquaticum [illegible]	0.049	0.045	0.048	0.031	0.049	0.045	0.045	0.038	0.032	0.031	0.023	0.045	0.041	0.035										
16 Chryseobacterium molle [illegible]	0.049	0.048	0.050	0.047	0.038	0.035	0.031	0.020	0.037	0.031	0.046	0.030	0.036	0.046	0.030									
17 Chryseobacterium pallidum [illegible]	0.050	0.049	0.056	0.050	0.048	0.034	0.042	0.027	0.037	0.034	0.043	0.034	0.040	0.051	0.038	0.024								
18 Chryseobacterium gleum [illegible]	0.051	0.052	0.061	0.055	0.047	0.048	0.043	0.037	0.043	0.039	0.043	0.046	0.008	0.042	0.041	0.038	0.046							
19 Chryseobacterium scophthalmum [illegible]	0.051	0.045	0.052	0.044	0.049	0.046	0.026	0.028	0.020	0.041	0.023	0.039	0.038	0.010	0.030	0.038	0.044	0.039						
20 Chryseobacterium daecheongense [illegible]	0.051	0.050	0.056	0.045	0.052	0.049	0.031	0.047	0.038	0.028	0.043	0.052	0.042	0.045	0.042	0.052	0.043	0.042	0.039					
21 Chryseobacterium vrystaatense [illegible]	0.052	0.053	0.057	0.051	0.060	0.058	0.049	0.046	0.046	0.055	0.043	0.052	0.031	0.038	0.050	0.052	0.055	0.031	0.041	0.051				
22 Chryseobacterium greenlandense [illegible]	0.052	0.048	0.048	0.035	0.052	0.043	0.042	0.038	0.031	0.032	0.027	0.045	0.040	0.039	0.035	0.038	0.038	0.045	0.029	0.043	0.052			
23 Chryseobacterium marinum [illegible]	0.052	0.048	0.055	0.044	0.023	0.052	0.044	0.047	0.052	0.054	0.049	0.056	0.046	0.051	0.050	0.048	0.052	0.046	0.053	0.050	0.061	0.052		
24 Amoebinatus massiliae [illegible]	0.052	0.049	0.054	0.043	0.042	0.042	0.049	0.037	0.039	0.038	0.034	0.037	0.050	0.042	0.029	0.035	0.035	0.050	0.040	0.051	0.051	0.031	0.051	
25 Elizabethkingia meningoseptica [illegible]	0.055	0.052	0.049	0.054	0.058	0.062	0.052	0.049	0.057	0.052	0.061	0.056	0.057	0.063	0.052	0.045	0.058	0.058	0.055	0.063	0.067	0.051	0.063	0.059
26 Chryseobacterium piperi [illegible]	0.053	0.049	0.051	0.046	0.052	0.052	0.050	0.052	0.039	0.045	0.042	0.060	0.048	0.044	0.042	0.056	0.049	0.046	0.046	0.049	0.050	0.042	0.056	0.049

- The range of differences in 16S rRNA sequences between *Chryseobacterium* species is .008 - .061.
- The difference between FH-1 and *E. lactis* 16S rRNA sequences is .021.

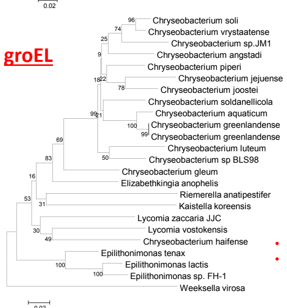
rpoB



Epilithimonas_sp_FH-1_rpoB																		
Epilithonimonas_lactis_DSM_19221T_rpoB_(JX293140)	0.024																	
Epilithonimonas_tenax_DSM_16811T_rpoB_(JX293141)	0.034	0.037																
Chryseobacterium_aquaticum_KCTC_12483T_rpoB_(JX293150)	0.137	0.146	0.145															
Chryseobacterium_soldanellicola_DSM_17072T_rpoB_(JX293155)	0.141	0.144	0.141	0.059														
Chryseobacterium_soli_DSM_19298T_rpoB_(JX293148)	0.141	0.151	0.148	0.076	0.090													
Chryseobacterium_joostei_LMG_18212T_rpoB_(JX293149)	0.141	0.151	0.149	0.058	0.055	0.072												
Chryseobacterium_luteum_DSM_18605T_rpoB_(JX293146)	0.144	0.149	0.148	0.069	0.061	0.076	0.061											
Chryseobacterium_angstadti_KMT_rpoB_(JX293142)	0.144	0.152	0.152	0.077	0.072	0.066	0.066	0.054										
Chryseobacterium_oranimense_LMG_24030T_rpoB_(JX293147)	0.148	0.158	0.161	0.079	0.068	0.089	0.068	0.068	0.062									
Chryseobacterium_greenlandense_UMB34T_rpoB_(JX293152)	0.148	0.159	0.158	0.023	0.062	0.080	0.061	0.061	0.075	0.080								
Chryseobacterium_shigense_DSM_17125T_rpoB_(JX293144)	0.149	0.155	0.154	0.070	0.068	0.056	0.061	0.059	0.062	0.068	0.073							
Chryseobacterium_novum_JM1_rpoB	0.151	0.154	0.155	0.085	0.083	0.069	0.066	0.058	0.051	0.073	0.085	0.059						
Chryseobacterium_greenlandense_UMB10_rpoB_(JX293151)	0.151	0.162	0.161	0.025	0.065	0.083	0.063	0.063	0.077	0.083	0.003	0.076	0.087					
Chryseobacterium_piperi_CTMT_rpoB_(JX293154)	0.152	0.161	0.156	0.083	0.089	0.083	0.075	0.087	0.066	0.083	0.083	0.072	0.086	0.086				
Chryseobacterium_novum_FH2_rpoB	0.152	0.161	0.162	0.096	0.072	0.104	0.082	0.090	0.086	0.079	0.097	0.094	0.093	0.100	0.077			
Chryseobacterium_vrystaatense_LMG_22846T_rpoB_(JX293143)	0.154	0.168	0.162	0.076	0.080	0.059	0.065	0.058	0.055	0.085	0.073	0.046	0.059	0.076	0.085	0.106		
Chryseobacterium_gleum_ATCC_35910T_rpoB_(GL379781)	0.158	0.166	0.162	0.094	0.087	0.101	0.079	0.079	0.085	0.097	0.100	0.101	0.099	0.103	0.079	0.096	0.099	
Chryseobacterium_jejuense_DSM_19299T_rpoB_(JX293153)	0.161	0.165	0.163	0.099	0.099	0.100	0.068	0.092	0.076	0.093	0.106	0.097	0.065	0.108	0.075	0.090	0.094	0.079

- The range of differences in rpoB sequences between *Chryseobacterium* species is .051 - .106.
- The difference between FH-1 and *E. lactis* rpoB sequences is .024.

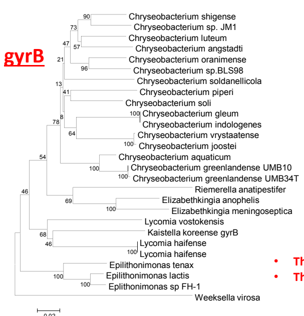
groEL



Epilithonimonas_sp_FH-1_groEL																
Epilithonimonas_lactis_DSM_19921_groEL	0.041															
Epilithonimonas_tenax_DSM_16811	0.084	0.082														
Chryseobacterium_gleum_ATCC_35910_groEL	0.184	0.179	0.166													
Chryseobacterium_sp_BLS98_groEL	0.192	0.184	0.159	0.105												
Chryseobacterium_aquaticum_KCTC_12483_groEL	0.192	0.189	0.179	0.128	0.105											
Chryseobacterium_sp_KM_groEL	0.194	0.194	0.174	0.136	0.102	0.110										
Chryseobacterium_soldanellicola_DSM_17072_groEL	0.197	0.194	0.166	0.128	0.084	0.084	0.087									
Chryseobacterium_haifense_DSM_groEL	0.199	0.187	0.184	0.153	0.176	0.176	0.202	0.179								
Chryseobacterium_greenlandense_UMB10_groEL	0.199	0.197	0.182	0.123	0.100	0.026	0.095	0.077	0.179							
Chryseobacterium_greenlandenseUMB34_groEL	0.199	0.197	0.182	0.123	0.100	0.026	0.095	0.077	0.179	0.000						
Chryseobacterium_joostei_LMG_18212_groEL	0.199	0.197	0.182	0.120	0.100	0.102	0.100	0.074	0.184	0.092	0.092					
Chryseobacterium_piperi_CTM_groEL	0.202	0.189	0.174	0.120	0.084	0.100	0.077	0.074	0.171	0.082	0.082	0.092				
Chryseobacterium_vrystaatense_LMG_22846_groEL	0.202	0.194	0.159	0.113	0.084	0.074	0.079	0.074	0.192	0.069	0.069	0.102	0.077			
Chryseobacterium_soli_DSM_19298_groEL	0.202	0.202	0.164	0.125	0.090	0.084	0.079	0.077	0.197	0.074	0.074	0.105	0.074	0.023		
Chryseobacterium_sp_JM1_groEL	0.207	0.210	0.182	0.136	0.107	0.105	0.087	0.120	0.199	0.097	0.097	0.115	0.095	0.064	0.066	
Chryseobacterium_luteum_DSM_18605_groEL	0.210	0.207	0.187	0.138	0.082	0.095	0.107	0.100	0.210	0.084	0.084	0.123	0.102	0.082	0.079	0.113

- The range of differences in groEL sequences between *Chryseobacterium* species is .069 - .210.
- The difference between FH-1 and *E. lactis* groEL sequences is .041.

gyrB



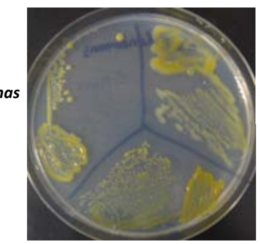
Epilithonimonas_diehli_FH1_gyrB																		
Epilithonimonas_lactis_DSM_19221T_gyrB_(JX293157)	0.030																	
Epilithonimonas_tenax_DSM_16811T_gyrB_(JX293158)	0.087	0.086																
Chryseobacterium_oranimense_LMG24030_gyrB	0.165	0.156	0.165															
Chryseobacterium_aquaticum_10-46_gyrB	0.167	0.165	0.169	0.116														
Chryseobacterium_greenlandense_UMB10_gyrB_(JX293161)	0.167	0.171	0.186	0.146	0.048													
Chryseobacterium_greenlandense_UMB34T_gyrB_(JX293162)	0.169	0.173	0.188	0.148	0.049	0.002												
Chryseobacterium_soldanellicola_DSM_17072_gyrB	0.175	0.179	0.190	0.118	0.116	0.143	0.144											
Chryseobacterium_luteum_strain_DSM_18605_gyrB	0.177	0.173	0.173	0.112	0.112	0.135	0.137	0.141										
Chryseobacterium_diehli_BLS98_gyrB	0.179	0.171	0.165	0.082	0.122	0.137	0.139	0.143	0.114									
Chryseobacterium_shigense_DSM_17126_gyrB	0.183	0.177	0.181	0.114	0.112	0.124	0.125	0.122	0.103	0.122								
Chryseobacterium_joostei_LMG_18212_gyrB	0.186	0.179	0.179	0.133	0.122	0.144	0.146	0.131	0.143	0.133	0.137							
Chryseobacterium_soli_DSM_19298_gyrB	0.188	0.177	0.183	0.120	0.114	0.122	0.124	0.144	0.116	0.118	0.118	0.125						
Chryseobacterium_vrystaatense_LMG_22846_gyrB	0.188	0.179	0.181	0.133	0.120	0.143	0.144	0.135	0.141	0.131	0.139	0.004	0.124					
Chryseobacterium_sp._JM1_gyrB	0.194	0.190	0.194	0.108	0.135	0.137	0.139	0.135	0.101	0.124	0.078	0.135	0.133	0.137				
Chryseobacterium_angstadti_KM_gyrB	0.196	0.194	0.188	0.116	0.133	0.150	0.152	0.139	0.097	0.125	0.108	0.146	0.124	0.143	0.101			
Chryseobacterium_piperi_CTM_gyrB	0.209	0.205	0.207	0.139	0.120	0.143	0.144	0.143	0.137	0.141	0.125	0.137	0.118	0.135	0.131	0.141		
Chryseobacterium_gleum_ATCC_35910_gyrB	0.213	0.209	0.202	0.148	0.131	0.143	0.144	0.152	0.139	0.143	0.141	0.124	0.143	0.120	0.144	0.129	0.143	
Chryseobacterium_indologenes_NCTC_10796_gyrB	0.213	0.209	0.202	0.148	0.131	0.143	0.144	0.152	0.139	0.143	0.141	0.124	0.143	0.120	0.144	0.129	0.143	0.000

- **The range of differences in gyrB sequences between *Chryseobacterium* species is .078 - .152.**
- **The difference between FH-1 and *E. lactis* gyrB sequences is .030.**

Phenotypes

Fatty Acid Methyl Ester	FH-1	E. lactis	E. tenax
iso-$C_{13:0}$	0.5	[illegible]	0.7
$C_{14:0}$	[illegible]	[illegible]	[illegible]
iso-$C_{15:0}$	[illegible]	[illegible]	15.1
anteiso-$C_{15:0}$	[illegible]	[illegible]	[illegible]
Summed Feature 2	0.4	[illegible]	[illegible]
Summed Feature 3	[illegible]	[illegible]	[illegible]
$C_{16:1}$ w5c	[illegible]	[illegible]	[illegible]
$C_{16:0}$	[illegible]	[illegible]	[illegible]
iso-$C_{15:0}$ 3-OH	[illegible]	2	[illegible]
$C_{15:0}$ 2-OH	[illegible]	[illegible]	[illegible]
Summed Feature 9	[illegible]	[illegible]	[illegible]
anteiso-$C_{17:1}$ w9c	[illegible]	[illegible]	[illegible]
iso-$C_{16:0}$ 3-OH	[illegible]	2	[illegible]
$C_{16:0}$ 3-OH	[illegible]	7.5	[illegible]
iso-$C_{17:0}$ 3-OH	[illegible]	[illegible]	[illegible]
$C_{17:0}$ 2-OH	ND	[illegible]	[illegible]

Fatty Acid Methyl Ester Composition of all three strains is similar



All three strains have distinct colony morphologies, produce yellow-orange flexirubin pigments

Biolog GenIII plates

Significant differences observed between strain FH-1 and E. lactis in utilization of:

- Trehalose
- Lactose
- Fructose
- Galactose
- Pectin
- Acetic Acid

In resistance to:

- Minocycline
- K-tellurite

	FH-1	E. lactis	E. tenax
neg control	22.7	21	20
dextrin	100	100	76
D-maltose	100	99.3	87
D-trehalose	13.7	99.3	73
D-cellobiose	15.7	21.7	16
gentiobiose	100	99	92
sucrose	14.3	15.7	93
D-turanose	11.3	12	8
stachyose	14.7	15.3	11
pos control	100	99.7	100
pH 6	99.7	99	32
pH 5	18	20.7	26
D-raffinose	17	20	15
a-D-lactose	16.3	99	16
D-melibiose	17.3	21	12
b-methyl-D-glucoside	15	16	11
D-salicin	100	99	9
N-acetyl-D-glucosamine	14	11.3	6
N-acetyl-b-D-mannosamine	15.7	11	6
N-acetyl-D-galactosamine	12	12.7	11
N-acetyl neuraminic acid	13	14.3	12
1% NaCl	74.3	98.7	14
4% NaCl	12.3	14.7	10
8% NaCl	23.7	30.7	49
a-D-glucose	99.7	99	86
D-mannose	10.3	10	88
D-fructose	99.3	17.3	8
D-galactose	15	98.7	10
3-methyl glucose	11.3	22.3	8
D-fucose	12	19.7	14
L-fucose	12	22.3	11
L-rhamnose	99	99	9
inosine	13.3	16.3	10
1% Na-lactate	99.3	98.3	32
fusidic acid	13	15	11
D-serine	17.7	19.3	13
D-sorbitol	16.7	20	15
D-mannitol	13.7	15.3	9
D-arabitol	11.7	13	6
myo-inositol	12.3	15.3	10
glycerol	11	11.3	8
D-glucose-6-PO4	20.7	18.3	100
D-fructose-6-PO4	14	20	7
D-aspartic acid	11	9.67	6
D-serine	11.3	9.33	6
troleandomycin	8	10	6
rifamycin SV	14	14	8
minocycline	41.3	19.3	13
gelatin	100	100	24
glycyl-L-proline	66	74.3	62
L-alanine	23.7	12	8
L-arginine	15.7	18	7
L-aspartic acid	24	19.7	6
L-glutamic acid	97	97	23
L-histidine	11.5	15	6
L-pyroglutamic acid	14	12	6
L-serine	11.3	10	6
lincomycin	10	11.7	5
guanidine HCl	12	16	6
niaproof 4	12.7	17	14
pectin	98.7	47.3	27
D-galacturonic acid	99	99	100
L-galacturonic acid lactone	11.3	11.3	6
D-gluconic acid	14	15.3	6
D-glucuronic acidi	18.3	18.7	6
glucuronamide	20.3	20.7	9
mucic acid	14.7	14.3	6
quinic acid	15.7	15.7	6
D-saccharic acid	13.3	13	7
vancomycin	10.3	15.7	7
tetrazolium violet	41.3	45.7	59
tetrazolium blue	70.3	100	80
p-hydroxy-phenylacetic acid	18.3	22.7	19
methyl pyruvate	65.7	91	12
D-lactic acid methyl ester	16.7	18	10
L-lactic acid	13.3	15.3	9
citric acid	15.3	14	6
a-keto-glutaric acid	14.3	15.7	6
D-malic acid	14.3	13.7	6
L-malic acid	18.7	15.7	6
bromo-succinic acid	10.3	11	6
nalidixic acid	20.3	16.7	7
LiCl	21	15.7	5
K-tellurite	70.3	42.7	14
tween-40	58	99	26
g-amino-butyric acid	16.3	16.7	12
a-hydroxy-butyric acid	13.7	16.3	12
b-hydroxy-D,L-butyric acid	15.7	17.3	9
a-keto-butyric acid	13.3	13.3	6
acetoacetic acid	15.3	22.3	7
propionic acid	17.7	17.3	6
acetic acid	55.3	98	65
formic acid	17	14.7	6
aztreonam	39.3	24	94
Na-butyrate	21.7	20	6
Na bromate	24	21	7

Conclusion

While phenotypic differences indicate that *Epilithonimonas sp.* FH-1 may be a novel species, the molecular evidence, specifically the percent differences of protein-coding genes between FH-1 and *Epilithonimonas lactis,* is inconclusive. DNA-DNA hybridization must be done to conclude whether FH-1 is a novel species.

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