Novel *Sphingobacteriaceae* Related to *Pedobacter agri* Isolated from a Freshwater Creek

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Abstract

As part of an undergraduate microbiology course, a pink pigmented, Gram-staining negative, rod-shaped, non-motile bacterial strain, designated R20-19 was isolated from a creek in north-central Pennsylvania during the summer of 2011. Comparative 16S rRNA sequences identified the closest match to several *Pedobacter* species. Full genome sequencing of the isolate and reference type strains revealed an approximately 80% average amino acid identity (AAI) relative to other pink-pigmented species *Pedobacter agri*, *Pedobacter ginsenosidimutans* and *Pedobacter borealis*, well below the 95% threshold for different species. Genomic comparison to the yellow-pigmented type species *Pedobacter heparinus*, revealed an Average Amino Acid Identity (AAI) below 70%, which suggests that these organisms should be in a separate genus. Based on the genotypic and phenotypic results of this study, strain R20-19 represents a novel species within the *Sphingoobacteriaceae*. Many *Pedobactetr* species are highly divergent from the type species suggesting that significant reclassification into new genera is required.

Table 1. Phylogenomic Matrices

	16s	ANI	ROSA		Estimated DNA-DNA Hybridization 1	2	3	4	5	6	7	8	9	10
Pedobacter fisheri R20-19^T				1		22.3	22.4	22.6	21.7	21.7	21.8	20.5	19.3	21.5
Pedobacter agri PB92^T	97.97	80.22	46.2	2	81.1		22.5	22.8	22.4	21.9	22.1	20.7	20.3	22.7
Pedobacter ginsenosidimutans KACC 14530^T	97.64	80.36	45.0	3	80.2	80.1		45.7	26.3	23.5	23.3	21	20.5	21.8
Pedobacter borealis DSM 19626^T	97.29	80.45	42.5	4	80.1	80.3	91.4		26.7	23.7	23.9	21.5	20.5	21.8
Pedobacter suwonensis DSM 18130^T	96.74	79.63	45.0	5	79.5	78.8	85.2	85.0		23.9	24.2	20.7	20.5	21.9
Pedobacter soli DSM 18609^T	95.67	79.68	43.6	6	79.2	80.3	83.3	83.7	82.2		56.4	20.6	19.5	19.3
Pedobacter kyungheensis KACC 16221^T	95.94	79.68	42.7	7	79.0	79.6	82.9	83.4	82.7	96.0		20.6	19.9	23.0
Pedobacter rhizosphaerae DSM 18610^T	96.14	78.84	42.7	8	77.9	78.5	78.5	78.9	77.6	78.4	77.8		20.1	22.4
Pedobacter heparinus DSM 2366^T	94.52	76.77	25.3	9	65.8	67.1	65.7	66.4	65.5	65.4	65.1	66.2		22.7
Pseudopedobacter saltans DSM 12145^T	90.35	76.03	18.7	10	58.0	58.1	57.6	57.8	57.7	57.2	57.0	57.8	59.0	
					Average Amino Acid Identity (AAI)									

Table 2. Biolog GENIII Results

		P. fisheri R20-19	P. agri	P. ginsenosid-imutans	P. borealis	P. kyungheensis	P.rhizosphaerae	P. roseus	P. heparinus			P.fisheri R20-19	P. agri	P. ginsenosid-imutans	P. borealis	P. kyungheensis	P.rhizosphaerae	P. roseus	P. heparinus
A01	neg control	21	27	20	16	17	21	15	19	E01	gelatin	97	96	98	99	99	98	98	17
A02	dextrin	99	99	98	100	98	99	98	39	E02	glycyl-L-proline	72	80	85	77	90	74	93	12
A03	D-maltose	97	97	96	99	97	97	97	79	E03	L-alanine	17	9	12	7	11	12	28	9
A04	D-trehalose	98	96	95	55	96	96	96	50	E04	L-arginine	11	10	12	8	11	48	11	11
A05	D-cellobiose	97	96	96	94	97	97	96	92	E05	L-aspartic acid	79	97	92	64	93	78	93	9
A06	gentiobiose	98	99	96	96	97	98	96	75	E06	L-glutamic acid	89	97	92	98	95	91	95	10
A07	sucrose	97	98	97	15	90	66	97	68	E07	L-histidine	8	17	12	13	17	12	22	9
A08	D-turanose	97	82	97	42	96	97	97	96	E08	L-pyroglutamic acid	9	7	9	8	10	8	9	9
A09	stachyose	96	22	96	16	95	97	95	15	E09	L-serine	38	21	18	9	27	37	73	9
A10	pos control	98	100	97	99	97	97	97	97	E10	lincomycin	10	9	92	12	12	11	12	94
A11	pH 6	97	99	96	97	96	96	96	96	E11	guanidine HCl	9	13	59	11	15	12	14	50
A12	pH 5	19	22	27	15	15	23	18	18	E12	niaproof 4	16	14	12	16	13	15	12	12
B01	D-raffinose	93	46	96	15	82	45	97	18	F01	pectin	68	97	22	21	21	19	47	17
B02	a-D-lactose	96	92	96	96	97	96	97	94	F02	D-galacturonic acid	96	96	94	13	95	97	95	99
B03	D-melibiose	96	80	96	97	97	97	96	95	F03	L-galacturonic acid lactone	96	7	96	10	96	98	95	99
B04	b-methyl-D-glucoside	96	68	97	18	95	96	96	47	F04	D-gluconic acid	11	7	10	11	12	11	13	10
B05	D-salicin	97	66	97	12	96	97	96	96	F05	D-glucuronic acidi	45	92	71	60	96	93	94	99
B06	N-acetyl-D-glucosamine	93	91	95	38	95	94	95	98	F06	glucuronamide	22	7	9	16	15	13	23	12
B07	N-acetyl-b-D-mannosamine	9	12	12	9	9	9	9	99	F07	mucic acid	8	7	9	6	9	8	10	9
B08	N-acetyl-D-galactosamine	8	49	90	81	86	95	90	96	F08	quinic acid	8	7	9	8	8	8	8	9
B09	N-acetyl neuraminic acid	10	17	11	7	96	97	95	99	F09	D-saccharic acid	8	7	9	8	9	8	10	9
B10	1% NaCl	63	22	94	57	94	94	92	91	F10	vancomycin	96	9	74	97	93	95	94	95
B11	4% NaCl	12	24	12	9	9	16	10	11	F11	tetrazolium violet	83	52	99	96	98	99	98	94
B12	8% NaCl	16	20	13	25	13	20	13	15	F12	tetrazolium blue	100	60	99	100	99	99	99	99
C01	a-D-glucose	97	98	96	100	97	97	97	93	G01	p-hydroxy-phenylacetic acid	10	8	12	16	16	12	15	13
C02	D-mannose	96	93	95	99	97	96	96	97	G02	methyl pyruvate	10	7	9	10	13	11	13	11
C03	D-fructose	75	83	96	53	86	39	96	97	G03	D-lactic acid methyl ester	9	7	9	14	12	10	12	11
C04	D-galactose	97	79	96	100	97	97	97	98	G04	L-lactic acid	10	7	10	10	13	8	15	9
C05	3-methyl glucose	8	7	10	6	7	11	8	10	G05	citric acid	8	7	9	10	10	8	10	9
C06	D-fucose	8	7	9	8	7	8	7	9	G06	a-keto-glutaric acid	8	7	9	8	10	8	11	9
C07	L-fucose	19	15	55	38	62	71	87	59	G07	D-malic acid	8	7	9	10	9	8	9	9
C08	L-rhamnose	92	69	95	68	92	97	93	17	G08	L-malic acid	95	9	94	9	10	8	10	9
C09	inosine	9	11	12	8	9	12	8	13	G09	bromo-succinic acid	87	7	34	7	10	9	10	10
C10	1% Na-lactate	93	95	91	31	94	91	94	89	G10	nalidixic acid	97	98	95	96	94	95	94	95
C11	fusidic acid	12	12	11	10	11	12	11	10	G11	LiCl	9	12	8	11	13	11	13	8
C12	D-serine	18	15	16	13	17	20	15	18	G12	K-tellurite	23	13	13	20	22	18	20	15
D01	D-sorbitol	14	19	15	12	13	15	12	92	H01	tween-40	44	83	25	25	39	29	64	26
D02	D-mannitol	8	9	10	10	11	9	9	95	H02	g-amino-butyric acid	9	7	10	16	15	10	15	11
D03	D-arabitol	8	10	9	7	9	8	8	9	H03	a-hydroxy-butyric acid	8	7	9	15	12	9	14	11
D04	myo-inositol	9	10	9	8	14	16	8	9	H04	b-hydroxy-D,L-butyric acid	8	7	9	12	13	8	13	10
D05	glycerol	8	7	9	6	7	8	7	9	H05	a-keto-butyric acid	8	7	9	12	9	8	9	9
D06	D-glucose-6-PO4	14	13	11	12	12	14	12	10	H06	acetoacetic acid	31	7	23	10	26	8	28	14
D07	D-fructose-6-PO4	25	14	15	9	15	14	28	11	H07	propionic acid	8	7	9	12	10	8	11	9
D08	D-aspartic acid	10	7	9	7	9	9	9	9	H08	acetic acid	41	30	26	17	20	23	43	13
D09	D-serine	21	7	9	6	8	12	16	9	H09	formic acid	8	7	9	11	10	8	10	10
D10	troleandomycin	10	9	10	12	11	12	12	9	H10	aztreonam	97	99	96	99	97	96	97	96
D11	rifamycin SV	11	11	10	21	12	13	13	93	H11	Na-butyrate	11	12	9	22	15	12	15	12
D12	minocycline	15	15	13	14	14	15	17	13	H12	Na bromate	10	12	9	18	18	11	16	10

Figure 1. Neighbor Joining Tree based on 16s rRNA sequences

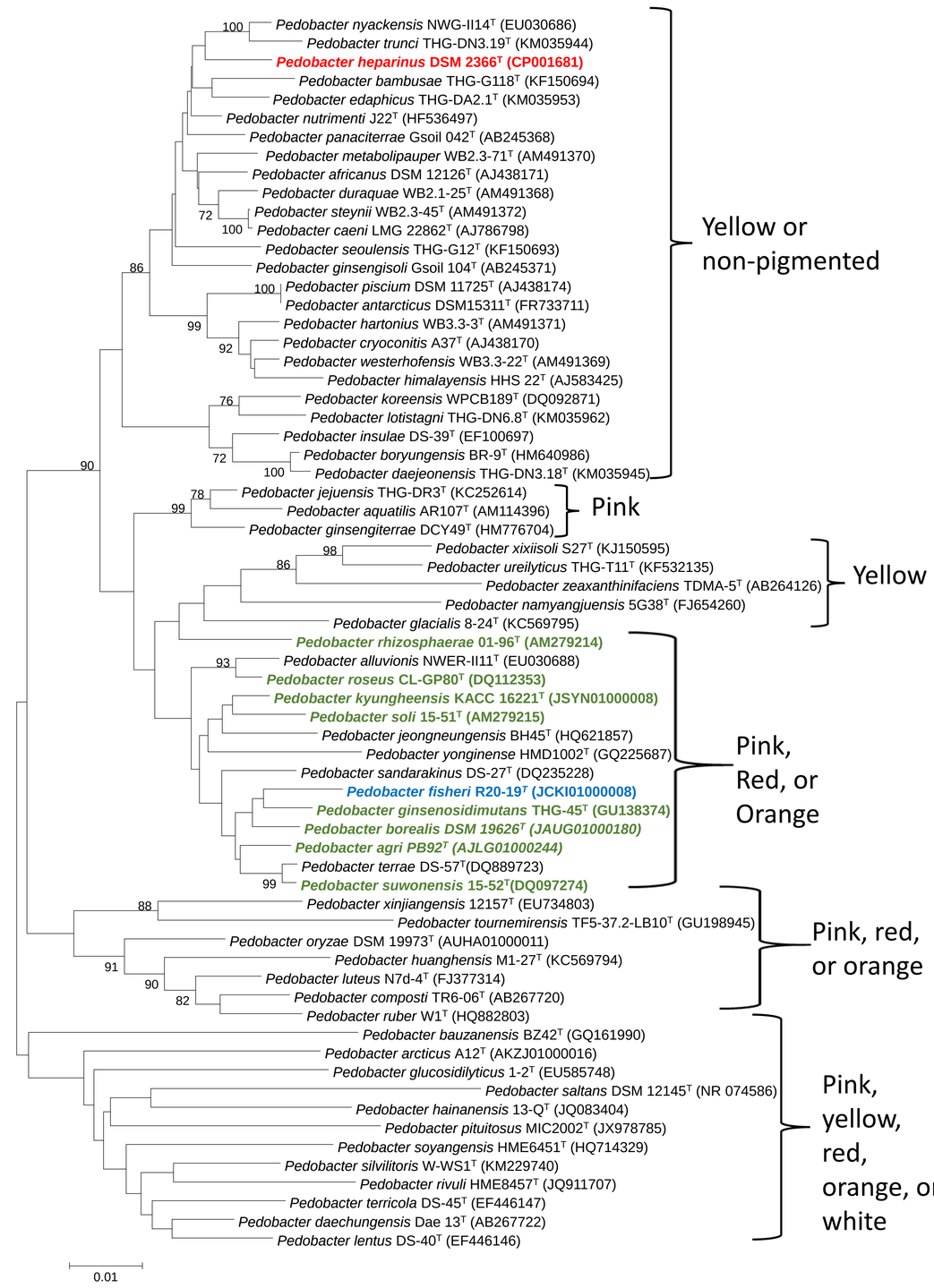


Table 3. MIDI Fame

fatty acid	P. fisheri	P. agri	P. ginseno-sidimutans	P. borealis	P. kyung-heensis	P. rhizo-sphaerae	P. heparinus
11:0 iso					1.2		
14:0 anteiso							1.1
14:0	1.3	1.5	1.7	1.0	1.2		1.3
SF1: 13:0 3OH/15:1 iso H	1.2		1.1		1		
15:0 iso	28.1	26.8	35.2	28.6	36.6	30.7	21.7
15:0 anteiso	2.2	2.2	1.1	1.6	1.1	1.3	1.9
15:1 w6c							1.4
SF3 16:1 w7c/w6c	26.5	23.3	14	17.4	20.3	18.5	23.7
SF3 16:1 w6c/w7c	7.9	10.4	13.9	17.5	5.4	13.7	7.1
16:1 w5c	3.3	3.9	1.3	1.9	2.8	2.7	2.2
16:0	3.5	2.6	1.5	3.1	2.5	2	3
15:0 iso 3OH	1.7	2.2	2.6	3	1.4	2	2.5
SF9 17:1 iso w9c/10 me 16:0	4.5	3	8	4.5	7.42	6.7	6.1
SF4 17:1 anteiso B/ 17:1 iso w5c (iso I)	1		1.6		1.8		1
17:1 anteiso w9c				2.2			2.4
17:1 w8c							1.3
16:0 iso 3OH	1.1		1.2		1.5		
16:0 3OH	1	3		1.6	1	1.2	1.1
17:0 iso 3OH	8	11.6	9.4	12.1	5.8	11.9	8.2
17:0 2OH							3

Figure 2. Venn Diagram of shared and unique genes

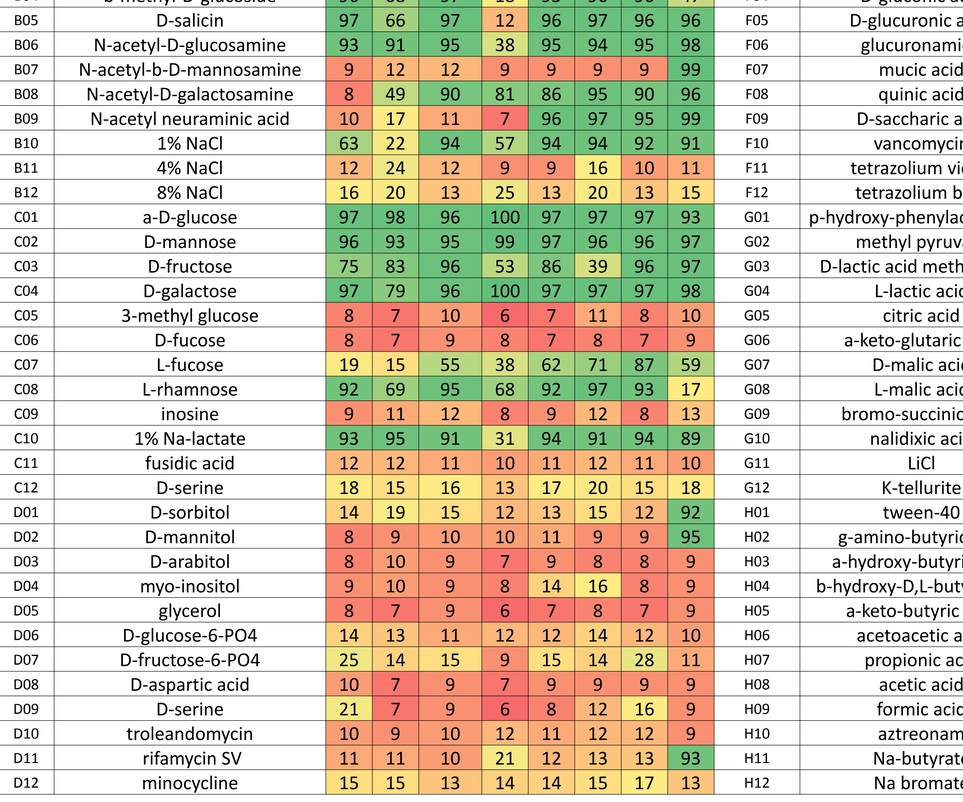
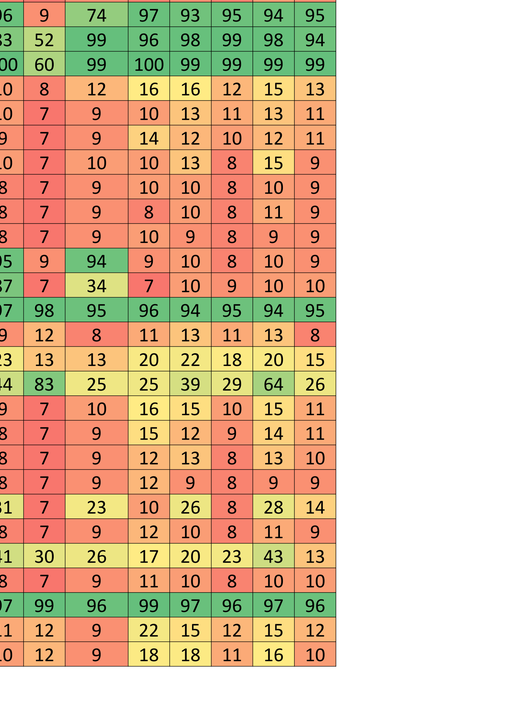


Image 1. Pigments of (1)*P. fisheri R20-19*, and reference organisms: (2)*P. agri (*3) *P. alluvionis* (4) *P. ginsenosidimutans (*5) *P. heparinus* (6) *P. borealis (*7) *P. eriksonii BMA* (8) *P. kyungheensis* on R2A plate.



Conclusion

- When compared to the reference organisms, there are enough differences in phenotypic and genetic characteristics for *Pedobacter fisheri R20-19* to be named a novel species.
- Based on the differences between *Pedobacter heparinus* and the reference organisms in Table 1, Table 2, Table 3, and Image 1, and the distance in the neighbor joining tree, the novel and reference organisms should be classified into a separate genus from *Pedobacter heparinus*.

References

- Kim, OS, Cho, YJ, Lee, K, Yoon, SH, Kim, M, Na, H, Park, SC, Jeon, YS, Lee, JH, Yi, H, Won, S, Chun, J. (2012). Introducing EzTaxon: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 62, 716–721.
- Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.-P., Göker, M. (2013). Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14:60.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. (2014). The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res.* 42:206-214.
- Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725-2729.