Characterization of *Chryseobacterium populense CF314 sp nov*

Authors: Victoria L. Bortniak[1], Jeffrey D. Newman[1]

[1] Biology Department, Lycoming College, 700 College Place, Williamsport, Pennsylvania, 17701

Abstract

Chryseobacterium sp nov CF314 was isolated from the rhizosphere of *Populus deltoides* growing alongside the Caney Fork river and Yadkin river in Tennessee and North Carolina and its genome was sequenced. An Eztaxon-e search of the 16S rRNA sequence yielded a 97.01% similarity to *Chryseobacterium daecheongense.* A bacterium is a potentially novel species when it has less that 98.5% similarity to published species. *C. sp CF314* will be compared to closely related species to determine phenotypic differences. The reference strains used are *Chryseobacterium daecheongense, Chryseobacterium wanjuense,* and *Chryseobacterium hispalense.* The name *Chryseobacterium populense* will be proposed.

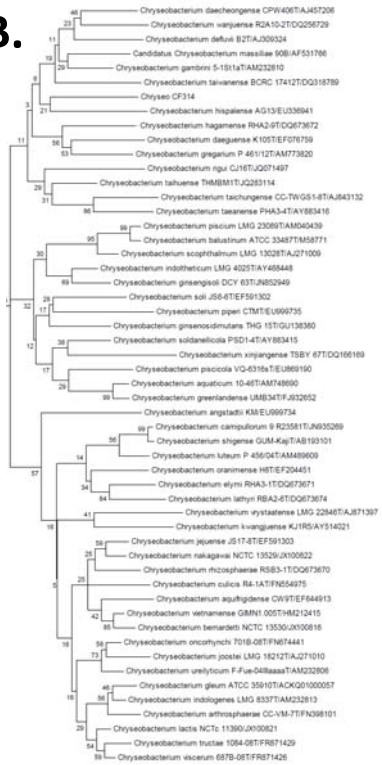
- While investigating Chryseobacterium genomes, the C. sp. CF314 genome was recognized as different from named species.
- To officially name a new species, it must be phenotypically characterized and compared to closest relatives.

A.

Rank	Name	Strain	Authors	Pairwise Identity (%)	DIF/Total et
1	Chryseobacterium daecheongense	CP0406(T)	Kim et al. 2005	97.01	42/1406
2	Chryseobacterium hispalense	AG13(T)	Montero-Calasanz et al. (in press)	96.80	45/1406
3	Chryseobacterium gleum	ATCC 35910(T)	(Holmes et al. 1984) Vandamme et al. 1994	96.73	46/1406
4	Chryseobacterium wanjuense	R2A10-2(T)	Woon et al. 2006	96.73	46/1406
5	Chryseobacterium arthrosphaerae	CC-VM-7(T)	Kämpfer et al. 2010	96.70	45/1364
6	Chryseobacterium miricolae	906	Greub et al. 2004	96.58	48/1406
7	Chryseobacterium taihuense	THMBM1(T)	Wu et al. 2013	96.51	48/1406
8	Chryseobacterium gwangjuense	THG-A18(T)	Park et al. (in press)	96.50	48/1401
9	Chryseobacterium indologenes	LMG 8337(T)	(Yabuuchi et al. 1983) Vandamme et al. 1994	96.48	48/1389
10	Chryseobacterium lactis	NCTC 11390(T)	Holmes et al. (in press)	96.49	48/1387
11	Chryseobacterium tructae	1084-08(T)	Zamora et al. 2013	96.48	48/1364
12	Chryseobacterium daeguense	K103(T)	Yoon et al. 2007	96.42	50/1387
13	Chryseobacterium scophthalmum	LMG 13028(T)	(Hudcarris et al. 1994) Vandamme et al. 1994	96.31	51/1383
14	Chryseobacterium piscicola	VQ-6316(T)	Bardi et al. 2009	96.24	52/1384
15	Chryseobacterium hagamense	RHA2-9(T)	Cho et al. 2011	96.23	53/1406
16	Chryseobacterium haifense	NF80(T)	Vaneechoutte et al. 2007	96.23	53/1405
17	Chryseobacterium carnipullorum	R_K2358(T)	Chermiba et al. (in press)	96.22	53/1403
18	Chryseobacterium yeoncheonense (invalid name)	SCY9(T)	Hoang et al. 2013	96.21	53/1397
19	Chryseobacterium defluvii	92(T)	Kämpfer et al. 2003	96.20	53/1393

Figure 1. Panel A. EzTaxon-e results with most similar 16S rRNA sequences. Panel B. Phylogenetic Tree (NJ) of *Chryseobacterium* genus.

B.



Based on pairwise similarity and phylogenetic tree, closest named species are

- *Chryseobacterium hispalense*
- *Chryseobacterium wanjuense*
- *Chryseobacterium deacheongense*

Figure 2. R2A Plate photo of *C. sp.CF314* and reference strains

Subsystems Identified by RAST Annotation of Genome (Aziz et al., 2008)

Aminosugars (0)

Di- and oligosaccharides (20)
- Maltose and Maltodextrin Utilization (7)
- Trehalose Uptake and Utilization (3)
- Lactose and Galactose Uptake and Utilization (8)
- Lactose utilization (2)

Organic acids (1) - Lactate utilization (1)

Sugar alcohols (10)
- Glycerol and Glycerol-3-phosphate Uptake and Utilization (8)
- Glycerol fermenation to 1,3-propanediol (2)

Polysaccharides (14)
- Glycogen metabolism (5)
- Cellulosome (9)

Monosaccharides (61)
- Mannose Metabolism (9)
- D-ribose utilization (3)
- Xylose utilization (12)
- Deoxyribose and Deoxynucleoside Catabolism (7)
- L-Arabinose utilization (4)
- D-Galacturonate and D-Glucuronate Utilization (26)

Resistance to antibiotics and toxic compounds (54)
- Cobalt-zinc-cadmium resistance (22)
- Multidrug Resistance, Tripartite Systems in Gram Negative Bacteria (5)
- Streptothricin resistance (1)
- Resistance to fluoroquinolones (4)
- Beta-lactamase (5)
- Multidrug Resistance Efflux Pumps (9)

abbrev	Cpop	Cpop	Cwan	Cwan	Cdae	Cdae	Chis
strain	CF314	CF314	DSM 17724	DSM 17724	DSM 15235	DSM 15235	DSM 25574
temp	4ºC	30ºC	4ºC	30ºC	4ºC	30ºC	4ºC
run by	TLB	TLB	TLB	TLB	TLB	TLB	TLB
13:0 iso	TR	2.49		1.34	TR	2.41	
14:0 iso					4.09	7.04	
15:0 iso	28.66	50.51	28.74	53.29	28.79	56.14	30.34
15:0 anteiso	2.12	0.98	4.34	TR	5.8	TR	6.4
16:0 iso	3.4	TR	1.81		5.15	TR	2.06
SF3 16:1 w7c/w6c	15.2	10.44	13.83	8.4	12.73	8.17	12.31
16:0	2.31	2.52	3.01	2.23	2.49	1.46	3.78
15:0 iso 3OH	2.65	3.63	2.44	3.52	2.54	2.63	2.79
SF9 17:1 iso w9c/ 10 me 16:0			24.13	10.8	17.52	6.43	22.55
17:1 anteiso w9c			TR		TR		1.26
17:0 iso	TR	1.21	TR	2.03		2	TR
16:0 iso 3OH	1.58	TR	TR		1.8		1.34
18:0		TR	1.13	TR			
17:0 iso 3OH	12.86	14.81	13.2	15.41	12.17	11.5	11.89
17:0 2OH	TR	TR	1.53		1.08	TR	1.7

Figure 3. Fatty Acid Methyl Ester Analysis - fatty acids comprising greater than 1% of total are listed.

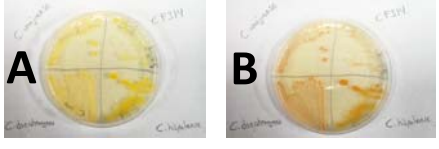


Figure 4. Plates before (A) and after (B) flooding with KOH to test for the production on flexirubin pigments.

A

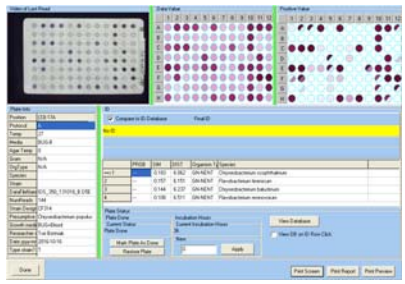


Figure 6. Biolog GenIII Plate
Panel A. Plate photo of BIOLOG results for CF314
Panel B. Detailed results of BIOLOG phenotypic tests

B

	Name	Chryseobacterium populense	Chryseobacterium hispalense	Chryseobacterium daecheongense	Chryseobacterium wanjuense		Name	Chryseobacterium populense	Chryseobacterium hispalense	Chryseobacterium daecheongense	Chryseobacterium wanjuense
A01	neg control	20	36	41	23	E01	gelatin	100	100	100	100
A02	dextrin	100	99	100	99	E02	glycyl-L-proline	99	93	97	96
A03	D-maltose	98	98	96	99	E03	L-alanine	28	21	6	3
A04	D-trehalose	97	11	95	99	E04	L-arginine	33	79	95	40
A05	D-cellobiose	14	14	52	48	E05	L-aspartic acid	93	98	99	90
A06	gentiobiose	95	97	95	99	E06	L-glutamic acid	99	99	99	98
A07	sucrose	11	13	97	19	E07	L-histidine	11	11	10	12
A08	D-turanose	11	34	21	10	E08	L-pyroglutamic acid	12	12	7	15
A09	stachyose	12	15	15	16	E09	L-serine	94	4	29	3
A10	pos control	93	95	97	94	E10	tetrazolium	92	7	94	92
A11	pH 6	94	94	97	95	E11	guanidine HCl	86	7	78	89
A12	pH 5	87	14	4	69	E12	niaproof 4	12	9	4	10
B01	D-raffinose	14	15	25	19	F01	pectin	20	32	53	23
B02	α-D-lactose	13	11	13	22	F02	D-galacturonic acid	98	99	44	97
B03	D-melibiose	11	27	25	35	F03	L-galacturonic acid lactone	8	5	3	6
B04	β-methyl-D-glucoside	12	13	18	16	F04	D-gluconic acid	16	20	13	23
B05	D-salicin	7	12	17	9	F05	D-glucuronic acid	25	30	27	37
B06	N-acetyl-D-glucosamine	8	12	16	16	F06	glucuronamide	31	40	28	30
B07	N-acetyl-β-D-mannosamine	11	14	15	18	F07	mucic acid	14	18	8	23
B08	N-acetyl-D-galactosamine	10	12	12	6	F08	quinic acid	14	20	7	18
B09	N-acetyl-neuraminic acid	8	5	6	9	F09	D-saccharic acid	10	8	3	7
B10	1% NaCl	91	91	94	92	F10	sienomycin	69	7	53	7
B11	4% NaCl	7	64	4	7	F11	tetrazolium violet	98	99	75	74
B12	8% NaCl	11	10	4	8	F12	tetrazolium blue	99	99	15	99
C01	α-D-glucose	83	92	86	98	G01	p-hydroxy-phenylacetic acid	12	14	11	8
C02	D-mannose	98	98	96	98	G02	methyl pyruvate	4	7	6	8
C03	D-fructose	99	98	24	99	G03	D-lactic acid methyl ester	17	16	9	18
C04	D-galactose	22	47	36	25	G04	L-lactic acid	12	11	8	31
C05	3-methyl glucose	13	21	21	21	G05	citric acid	23	30	45	31
C06	D-fucose	19	16	32	21	G06	α-keto-glutaric acid	14	17	9	94
C07	L-fucose	20	21	27	27	G07	D-malic acid	15	9	7	18
C08	L-rhamnose	92	97	29	96	G08	L-malic acid	16	24	11	20
C09	inosine	10	12	5	11	G09	bromo-succinic acid	9	4	1	3
C10	1% Na-lactate	90	88	89	92	G10	nalidixic acid	85	8	4	7
C11	fusidic acid	6	7	4	7	G11	LiCl	6	83	4	7
C12	D-serine	8	11	7	7	G12	K-tellurite	95	96	98	96
D01	D-sorbitol	13	18	30	21	H01	tween-40	91	98	77	96
D02	D-mannitol	15	14	13	18	H02	γ-amino-butyric acid	15	6	14	20
D03	D-arabitol	10	14	14	14	H03	α-hydroxy-butyric acid	18	13	8	20
D04	myo-inositol	13	13	22	21	H04	D-hydroxy-(L)-butyric acid	20	8	9	33
D05	glycerol	97	8	95	98	H05	α-keto-butyric acid	21	9	4	11
D06	D-glucose-6-PO4	16	31	13	25	H06	acetoacetic acid	95	97	97	13
D07	D-fructose-6-PO4	26	38	26	37	H07	propionic acid	38	11	22	11
D08	D-aspartic acid	9	4	1	4	H08	acetic acid	99	98	99	99
D09	D-serine	9	4	3	4	H09	formic acid	15	6	70	8
D10	troleandomycin	9	83	51	59	H10	rotenoxen	93	94	94	94
D11	rifamycin SV	91	86	93	92	H11	Na-butyrate	81	29	35	12
D12	minocycline	10	11	40	17	H12	Na-bromate	13	10	54	12

Conclusions

- 16S rRNA sequence suggests C. sp.CF314 is a novel species.
- FAME profile is consistent with *Chryseobacterium* species.
- Biolog data is consistent with genome sequence.
- C. sp CF314 can be phenotypically distinguished from closest relatives using Biolog GenIII plates.

Future Work

Finish testing and comparisons needed to publish *C. populense.*

References

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- Kim, O.S., *et al*. (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 62: 716–721