

Characterization of *Chryseobacterium populense* CF314 sp nov

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Abstract

Chryseobacterium sp. CF314 was isolated from the endosphere of *Populus deltoides* growing alongside the Caney Fork River and Yadin River in Tennessee and North Carolina and its genome was sequenced using Illumina HiSeq2000 technology (Brown et al., 2012). The 16S rRNA sequence is 97.0-96.7% similar to *Chryseobacterium daecheongense*, *Chryseobacterium hispalense*, and *Chryseobacterium wanjuiense*. To identify distinguishing characteristics, a series of phenotypic tests were performed, including Biolog GenIII plates, API ZYM and ZONE galleries, fatty acid methyl ester, polar lipid and pigment analysis. Because strain CF314 clustered most closely with *C. hispalense*, the *C. hispalense* genome was sequenced for comparative genomic studies instead of performing DNA-DNA hybridization. *Chryseobacterium* sp. CF314 is a gram negative, yellow pigmented rod. It grows in the temperature range 20-37°C, pH range 5.0-9.0, and 1% NaCl. The major fatty acids present are 15:0 iso, 17:0 iso 3OH, and 16:1ω6c/16:1ω7c. The average amino acid identity (AAI) between strain CF314 and *C. hispalense* was 80.1%, well below the 95% species threshold. Based on phenotypic and genomic uniqueness, *Chryseobacterium* sp. CF314 merits description as a novel species. The name *Chryseobacterium populense* sp. nov. will be proposed.

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

- *Chryseobacterium hispalense*
- *Chryseobacterium wanjuiense*
- *Chryseobacterium daecheongense*

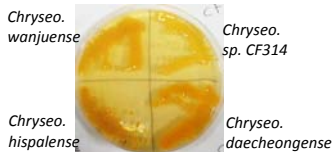


Figure 2. R2A Plate of *C. sp. CF314* and reference strains.

	<i>C. populense</i>	<i>C. hispalense</i>	<i>C. wanjuiense</i>	<i>C. daecheongense</i>
strain	CF314 ^a	DSM 25574 ^a	DSM 17724 ^a	DSM 15235 ^a
temp	30 C	30 C	30 C	30 C
13:0 iso	0.49	0.66	0.22	3.05
14:0	0.62	0.41	1.33	0.74
15:0 iso	43.21	51.6	49.82	62.29
15:0 anteiso	0.45	1.21	0.31	0.89
SF3 16:1 ω6c/ω7c	15.87	6.73	14.05	10.63
16:0	1.32	1.11	1.06	1.3
15:0 iso 3OH	5.42	3.55	7	3.37
SF9 17:1 iso ω9c/10 iso 16:0	4.48	21.49	2.32	6.8
17:0 iso	0.85	0.61	0.5	1.19
16:0 iso 3OH	0.52	0.68	1	0.37
16:0 3OH	1.37			
17:0 iso 3OH	23.79	8.55	19.37	7.49

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

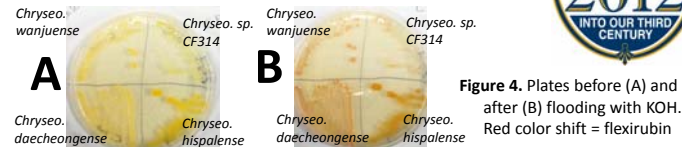


Figure 4. Plates before (A) and after (B) flooding with KOH. Red color shift = flexirubin

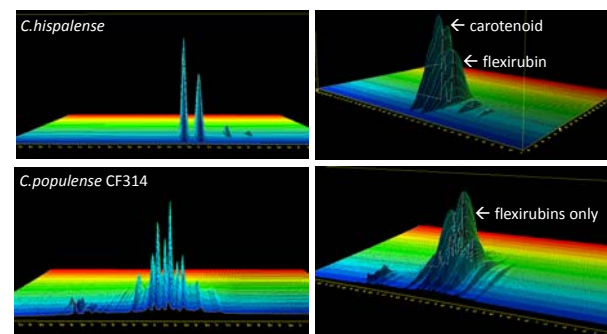


Figure 5. Pigment landscape and ortholog comparison.

C. hispalense produces carotenoids and flexirubins, *C. populense* produces flexirubins only.

<i>C. hispalense</i>		<i>C. populense</i>	
Gene	Function	Gene	Function
...
...
...
...

A. EzTaxon-e results with most similar 16S rRNA sequences.

Rank	Strain	Accession	Similarity (%)	Length (nt)
1	<i>Chryseobacterium daecheongense</i>	CPH607	97.02	152396
2	<i>Chryseobacterium hispalense</i>	NC0187	96.86	152396
3	<i>Chryseobacterium wanjuiense</i>	NC0187	96.75	152396
4	<i>Chryseobacterium daecheongense</i>	DSM 15235	96.72	152396
5	<i>Chryseobacterium hispalense</i>	DSM 25574	96.72	152396

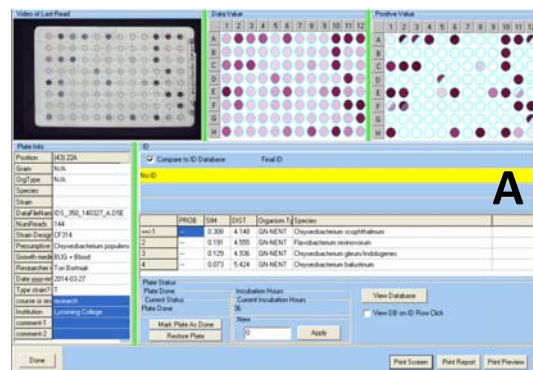


Figure 3. Biolog GenIII plate results and correlation to genome

Panel A. Plate photo of GenIII results for CF314

Panel B. Detailed comparison of GenIII plate results with reference strains.

- shaded reagents compared to positive control to detect growth inhibition.

Panel C. RAST (Aziz et al, 2008) annotations of Carbohydrate metabolism subsystems (“Central Metabolism” not shown) in *C. populense* CF314 and *C. hispalense* genomes.

B. Comparison of RAST subsystems between *C. populense* and *C. hispalense*.

Subsystem	<i>C. populense</i>	<i>C. hispalense</i>
Amisogars (7)	7	7
Di- and oligosaccharides (29)	29	29
Maltose and Maltodextrin Utilization (13)	13	13
Trehalose Uptake and Utilization (6)	6	6
Lactose and Galactose Uptake and Utilization (8)	8	8
Lactose utilization (2)	2	2
Glycoside Hydrolases (40)	40	40
One-carbon Metabolism (40)	40	40
Serine glyoxylate cycle (9)	9	9
One-carbon metabolism by tetrahydropterins (3)	3	3
Organic acids (5)	5	5
Glycerate metabolism (4)	4	4
Lactate utilization (1)	1	1
Fermentation (34)	34	34
Butanol Biosynthesis (14)	14	14
Acetolactate synthase subunits (2)	2	2
Acetyl-CoA fermentation to Butyrate (15)	15	15
Aerobic autotrophic metabolism (3)	3	3
CO2 fixation (0)	0	0
Sugar alcohols (8)	8	8
Glycerol and Glycerol-3-phosphate Uptake and Utilization (8)	8	8
Carbohydrates - no subcategory (0)	0	0
Polysaccharides (18)	18	18
Glycogen metabolism (5)	5	5
Cellulose (1)	1	1
Monosaccharides (62)	62	62
Mannose Metabolism (8)	8	8
D-ribose utilization (3)	3	3
Xylose utilization (10)	10	10
Decarboxylase and Deoxy nucleoside Catabolism (7)	7	7
Glycogen metabolism (5)	5	5
Cellulose (1)	1	1
D-Galacturonate and D-Glucuronate Utilization (29)	29	29

Chryseobacterium populense RAST subsystems
 Aminosugars (7)
 • N-Acetyl-Galactosamine and Galactosamine Utilization (7)
 Di- and oligosaccharides (29)
 • Maltose and Maltodextrin Utilization (13)
 • Trehalose Uptake and Utilization (6)
 • Lactose and Galactose Uptake and Utilization (8)
 • Lactose utilization (2)
 Glycoside Hydrolases (40)
 One-carbon Metabolism (40)
 • Serine glyoxylate cycle (9)
 • One-carbon metabolism by tetrahydropterins (3)
 Organic acids (5)
 • Glycerate metabolism (4)
 • Lactate utilization (1)
 Fermentation (34)
 • Butanol Biosynthesis (14)
 • Acetolactate synthase subunits (2)
 • Acetyl-CoA fermentation to Butyrate (15)
 • Aerobic autotrophic metabolism (3)
 CO2 fixation (0)
 Sugar alcohols (8)
 • Glycerol and Glycerol-3-phosphate Uptake and Utilization (8)
 Carbohydrates - no subcategory (0)
 Polysaccharides (18)
 • Glycogen metabolism (5)
 • Cellulose (1)
 Monosaccharides (66)
 • Mannose Metabolism (11)
 • D-ribose utilization (3)
 • Xylose utilization (10)
 • Decarboxylase and Deoxy nucleoside Catabolism (7)
 • Lactulose utilization (4)
 • L-arabinose utilization (4)
 • D-Galacturonate and D-Glucuronate Utilization (29)

Chryseobacterium hispalense RAST subsystems
 Aminosugars (0)
 Di- and oligosaccharides (21)
 • Maltose and Maltodextrin Utilization (8)
 • Lactose and Galactose Uptake and Utilization (9)
 • Lactose utilization (4)
 Trehalose Uptake and Utilization (6)
 • Lactose and Galactose Uptake and Utilization (8)
 • Lactose utilization (2)
 Glycoside Hydrolases (40)
 One-carbon Metabolism (38)
 • Serine glyoxylate cycle (34)
 • One-carbon metabolism by tetrahydropterins (4)
 Organic acids (1)
 • Lactate utilization (1)
 Fermentation (31)
 • Butanol Biosynthesis (16)
 • Acetyl-CoA fermentation to Butyrate (15)
 CO2 fixation (1)
 • CO2 uptake, carboxyome (1)
 Sugar alcohols (0)
 Carbohydrates - no subcategory (0)
 Polysaccharides (19)
 • Glycogen metabolism (5)
 • Cellulose (1)
 Monosaccharides (62)
 • Mannose Metabolism (8)
 • D-ribose utilization (3)
 • Xylose utilization (10)
 • Decarboxylase and Deoxy nucleoside Catabolism (7)
 • L-arabinose utilization (4)
 • D-Galacturonate and D-Glucuronate Utilization (80)

Phylogenomic Metrics (species threshold)	<i>C. populense</i>	<i>C. hispalense</i>
Genome size	4,488,346 bp	4,363,762 bp
GC mol% =	36.6%	36.7%
GGDC estimated DDH (70%) =		22%
Average Nucleotide Identity (ANI) (95%) =		77.8%
Average Amino Acid Identity (AAI) (95%) =		80.3%
Bidirectional Best Hits (%BBH) =		70.0 – 72.8%
Reciprocal Orthology Score Average (ROSA) (65%) =		46.1

Conclusion: *Chryseobacterium populense* CF314 sp. nov is sufficiently different from its closest relatives to merit description as a new species.

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