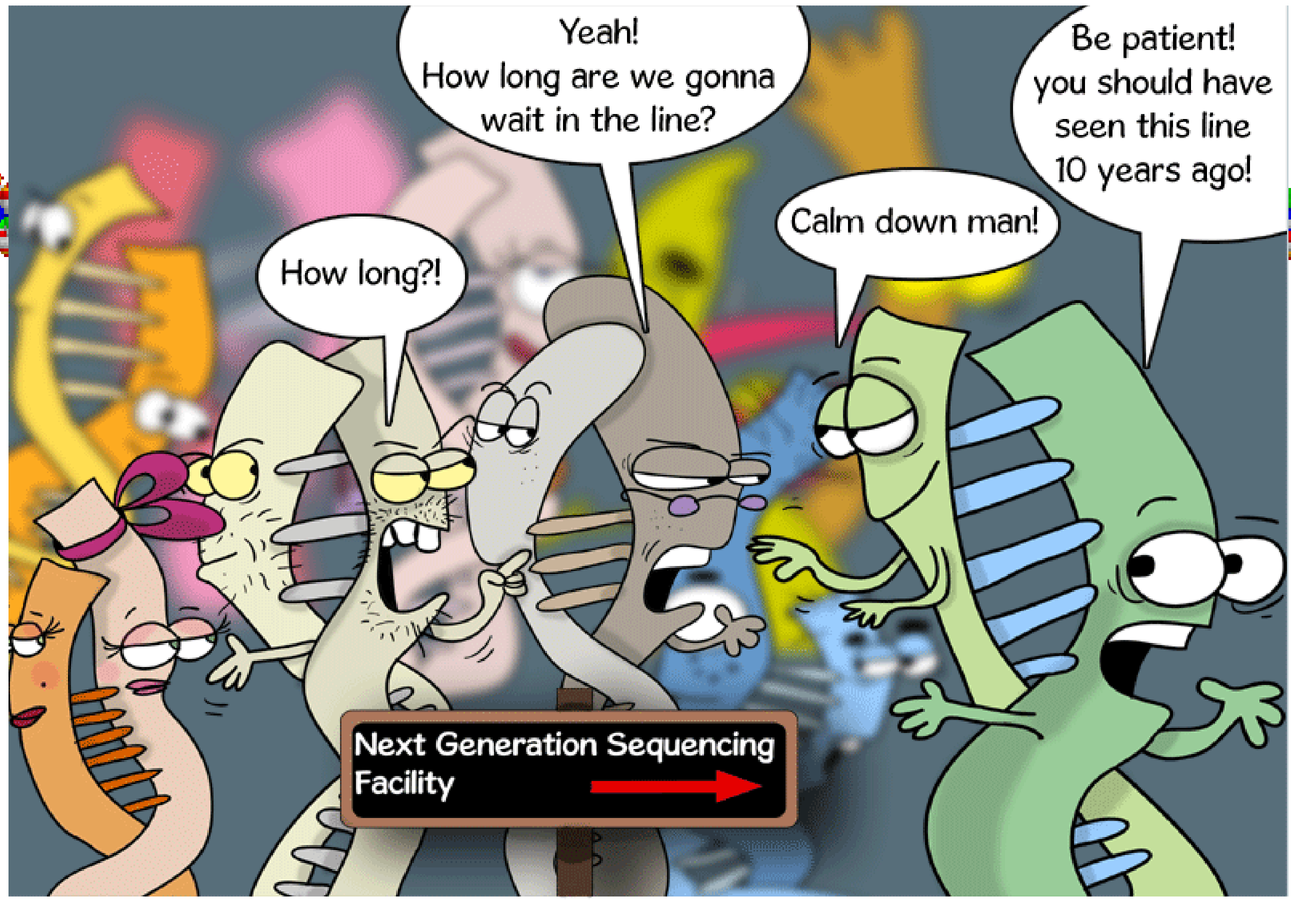
A Framework for Publishing Novel Bacterial Species Discovered in the Undergraduate Microbiology Lab

Jeff Newman

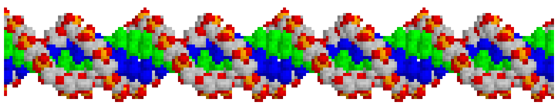
Lycoming College, Williamsport PA

June 17, 2016

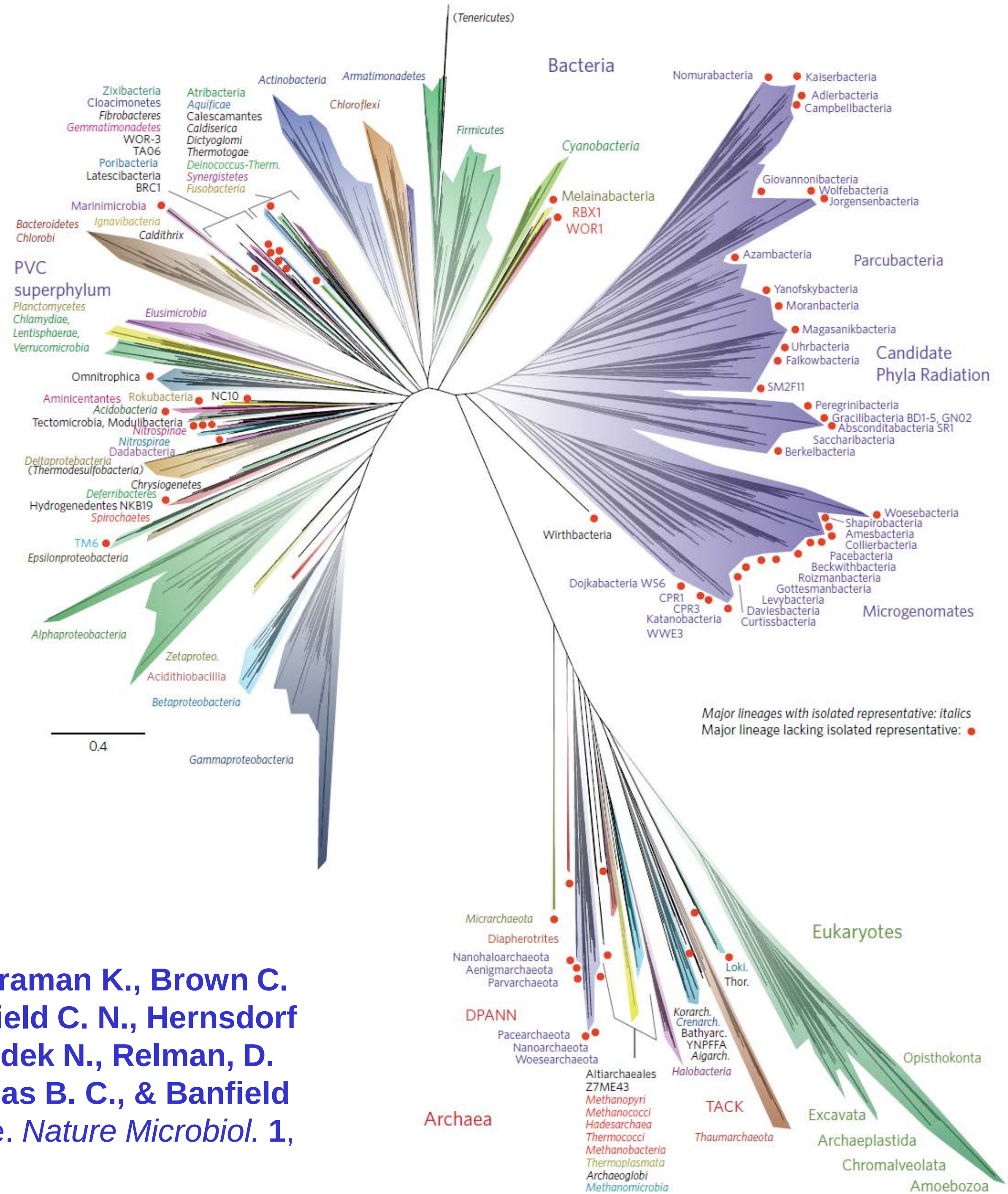


Human Genome 10th Anniversary

http://biocomicals.blogspot.com



The Tree of Life is Broad!



From Hug L. A., Baker B. J., Anantharaman K., Brown C. T., Probst A. J., Castelle C. J., Butterfield C. N., Hernsdorf A. W., Amano Y., Ise K., Suzuki Y., Dudek N., Relman, D. A., Finstad K. M., Amundson R., Thomas B. C., & Banfield J. F. (2016). A new view of the tree of life. *Nature Microbiol.* **1**, 16048.

The Tree of Life is DEEP!

Scaling laws predict global microbial diversity

Kenneth J. Locey[a,1] and Jay T. Lennon[a,1]

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Edited by David M. Karl, University of Hawaii, Honolulu, HI, and approved March 30, 2016 (received for review October 27, 2015)

Scaling laws underpin unifying theories of biodiversity and are among the most predictively powerful relationships in biology. However, scaling laws developed for plants and animals often go untested or fail to hold for microorganisms. As a result, it is unclear whether scaling laws of biodiversity will span evolutionarily distant domains of life that encompass all modes of metabolism and scales of abundance. Using a global-scale compilation of ~35,000 sites and ~5.6·10^6 species, including the largest ever inventory of high-throughput molecular data and one of the largest compilations of plant and animal community data, we show similar rates of scaling in commonness and rarity across microorganisms and macroscopic plants and animals. We document a universal dominance scaling law that holds across 30 orders of magnitude, an unprecedented expanse that predicts the abundance of dominant ocean bacteria. In combining this scaling law with the lognormal model of biodiversity, we predict that Earth is home to upward of 1 trillion (10^{12}) microbial species. Microbial biodiversity seems greater than ever anticipated yet predictable from the smallest to the largest microbiome.

hold across genomes, cells, organisms, ar
greatly varying size (13–15). Among the mos
the scaling of metabolic rate (B) with body s
(13)] and the rate at which species richne
species; S) scale with area [A; $S = cA^z$ (16)].
are predicted by powerful ecological theories
suggests that they fail for microorganisms (1
and body size, there is an equally general
diversity, that is, the number of individuals in
Often referred to as total abundance, N can r
10 individuals in a given area to the nearly 1
and archaea on Earth (6, 7). This expanse ou
of magnitude that separate the mass of
cell (3·1^{-16} kg) from a blue whale (1.9·10^5 kg)
magnitude that result from measuring Earth
spatial grain equivalent to bacteria (5.1·10^{26} μr

Here, we consider whether N may be one c
constraints on commonness and rarity and c

From Locey, K.J. and Lennon J.T. (2016) Scaling laws predict global microbial diversity. *Proc Natl Acad Sci USA* 113:5970-5975.

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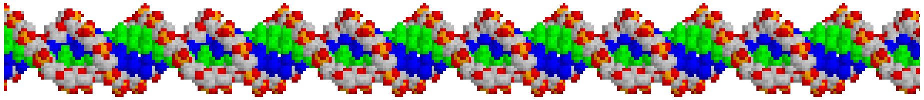
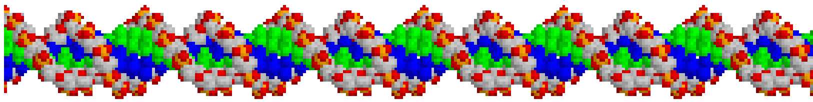
The Journey

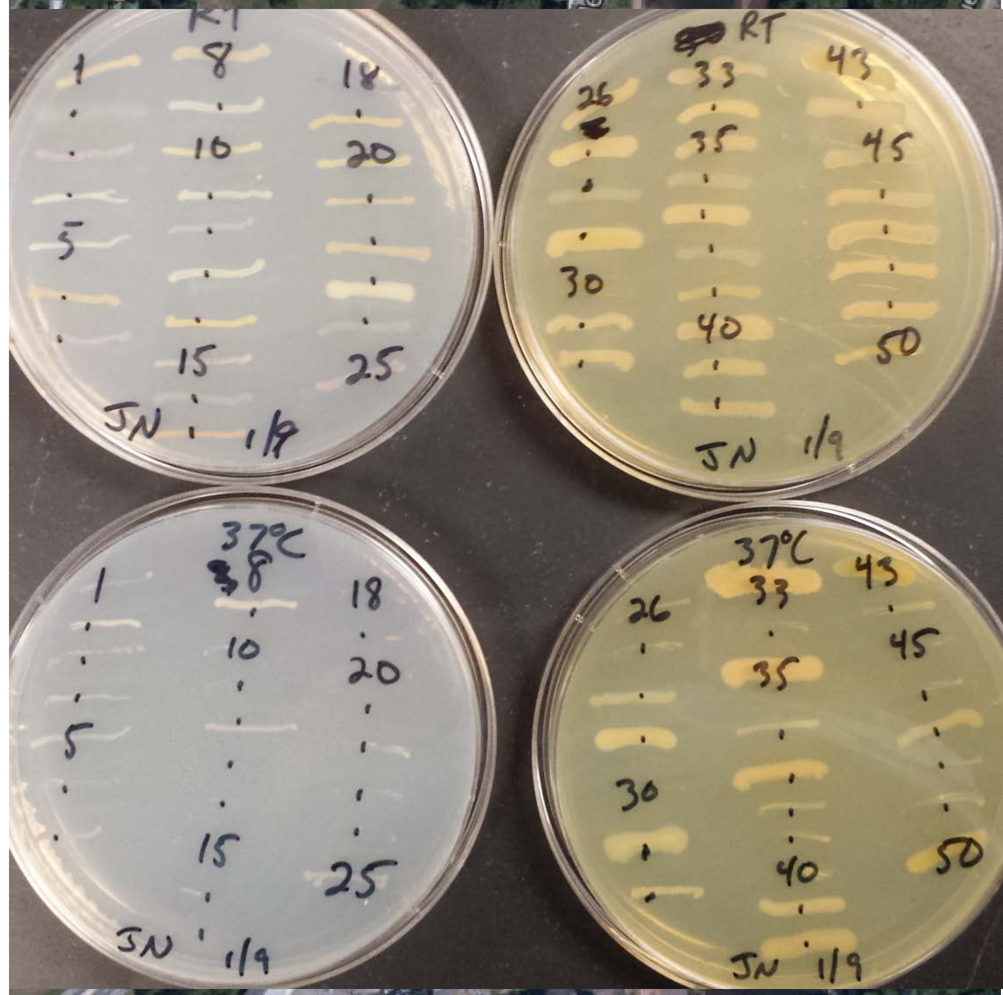


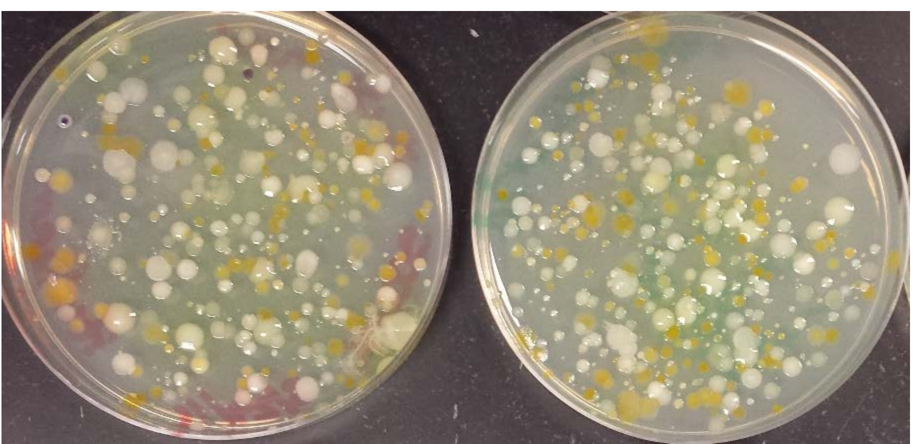
- Starting point: CURE - Micro UK's from freshwater creeks, 16S seq → novel species
- Compare phenotypes to closest type strains
  - Literature → family/genus specific tests
- Minimal Standards
- Best Practices: DDH→MLST→Genomes
- Calculate Phylogenomic Metrics (GGDC, ANI, AAI, ROSA)
- RAST/SEED viewer for genome analysis, correlation with phenotypes
- Simple web/excel tools use RAST output to:
  - Calculate AAI, BBH, ROSA
  - ID unique and shared genes
  - Create Venn Diagram
- Publish new genera & species!

Prep for Micro Lab

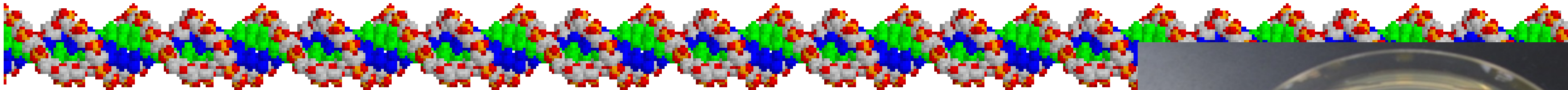


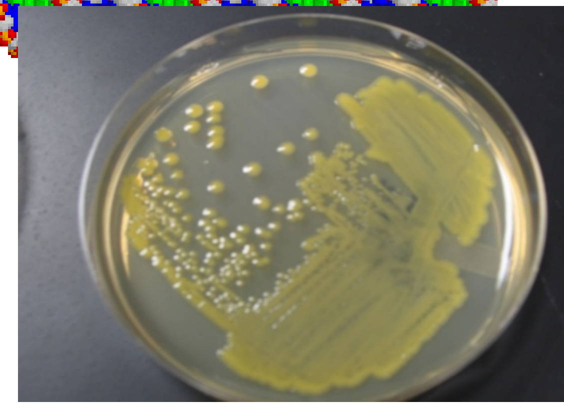
- Collect water + sediment sample late Dec. for Spring course, spread onto plates.
- Incubate @ RT 2-3 days
- Pick colonies, replicate patches @ RT & 37°C
- For safety – Patches that do NOT grow @ 37°C are streaked as unknowns





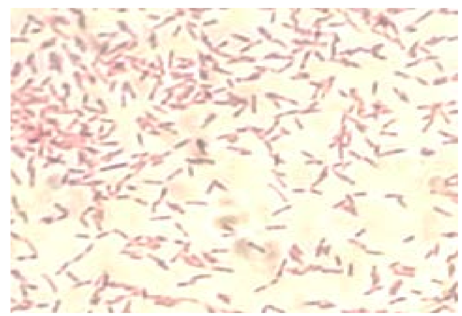
The LycoMicro Unknown Microbe Lab

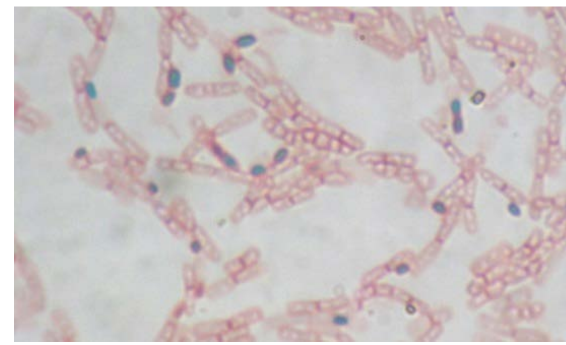




Week 1 - Aseptic technique/Inoculation

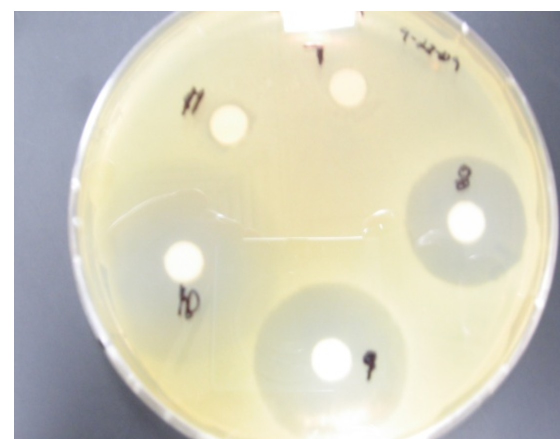
- pipetting sterile media
- selection of knowns and unknowns
- streak plates, inoculation of liquid
- preparation of frozen permanents





Week 2 – Staining & Microscopy

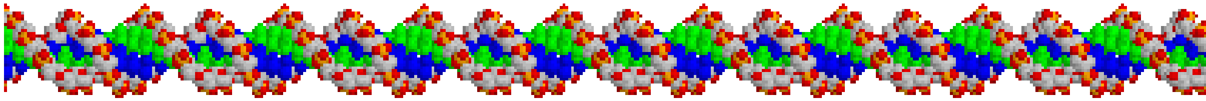
- Gram stain
- Endospore stain
- Wet mount

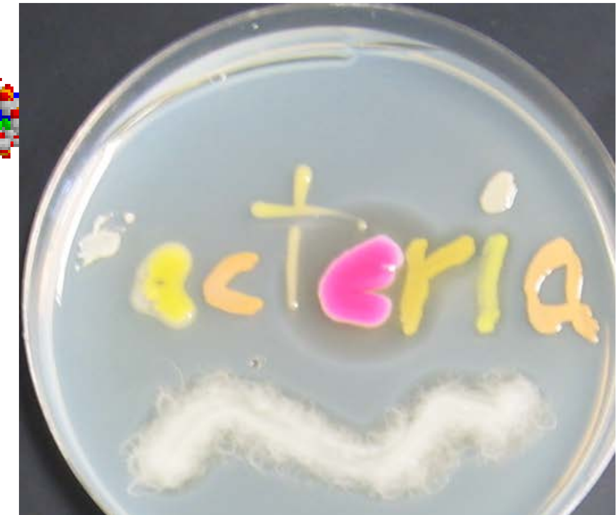


Week 3 – Antibiotic sensitivity, pH, [NaCl], Temperature, Oxygen requirements

- Kirby-Bauer Disk Diffusion assay (10)
- oxidase, catalase, Gas-Pak Jar

The LycoMicro Unknown Microbe Lab





Week 4 – Carbohydrate & Nitrogen Metabolism

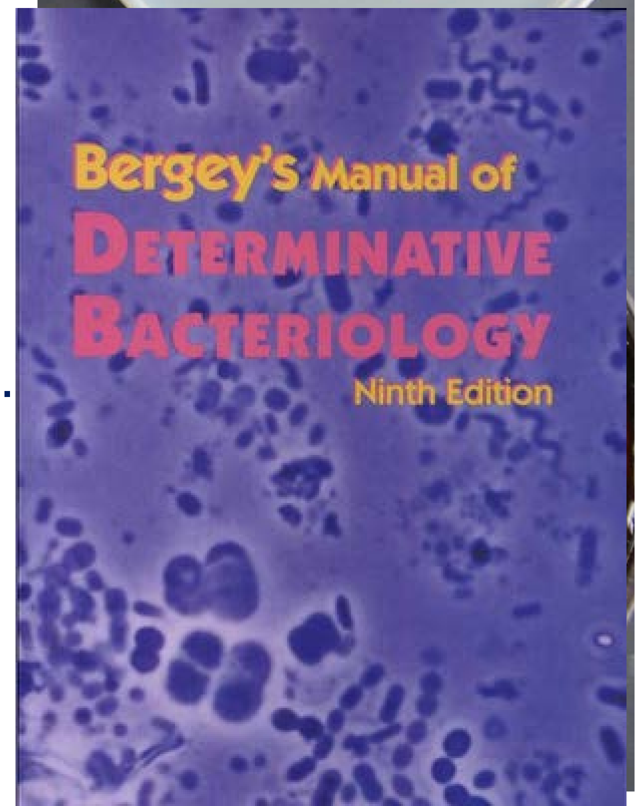
- MRVP, citrate, phenol red, TSI
- urease, nitrate reduction, SIM

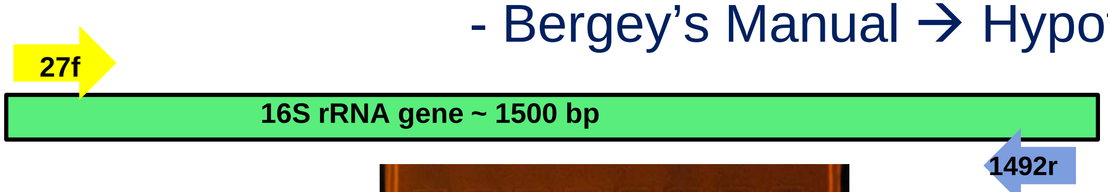
Week 5 – Exoenzymes, Differential/Selective media

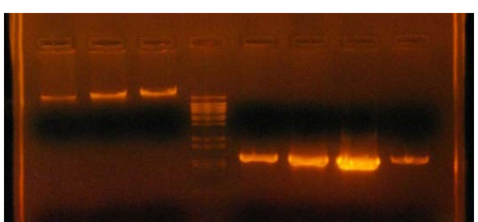
- caseinase, lipase, amylase, DNase,
- EMB, HEA, MSA, PhenylEthanol,
- Bile Esculin, Brilliant Green, EG Min.

Week 6 - PCR of 16S rDNA, gel, send for sequencing.

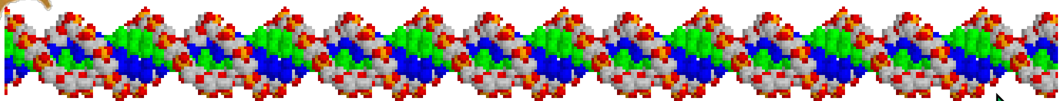
- Bergey’s Manual → Hypothesis







The LycoMicro Unknown Microbe Lab

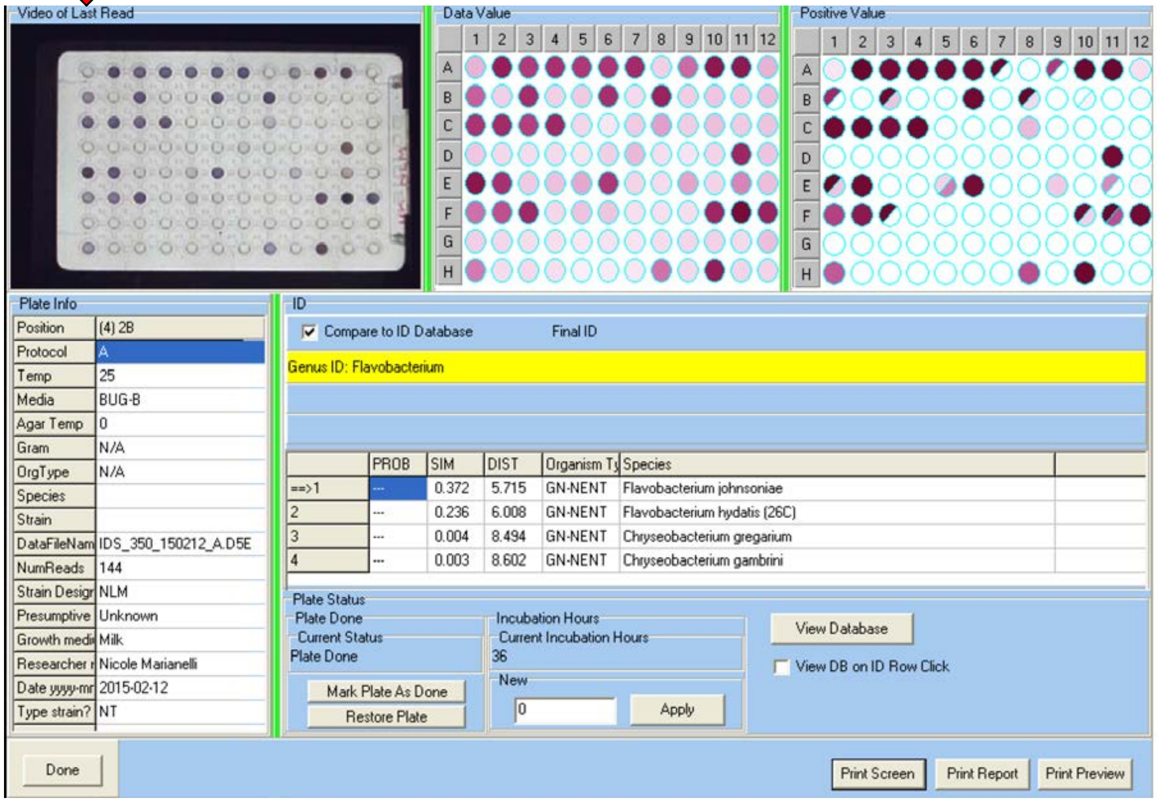


Week 7 – FAME Analysis

-Biolog GenIII Plates

(BUG+Blood also tests Hemolysis)

Databases give possible ID



Sherlock Sample Report E16224404A

Volume: DATA File: E162244.04A Samp Ctr: 6 ID Number: 2409
Type: Samp Bottle: 5 Method: ITSA1
Created: 2/24/2016 10:23:35 AM
Sample ID: unkn-CLH

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
0.7164	2.351E+9	0.025	----	6.5930	SOLVENT PEAK	----	< min rt	
2.1750	384	0.010	1.000	13.9156	14:1 w5c	0.14	ECL deviates 0.000	
2.1995	2462	0.009	0.997	13.9993	14:0	0.87	ECL deviates -0.001	Reference 0.005
2.3351	703	0.009	0.982	14.4397	15:1 iso F	0.25	ECL deviates -0.002	
2.3544	1655	0.009	0.980	14.5022	Sum In Feature 1	0.58	ECL deviates -0.001	13:0 3OH/15:1 iso H
2.3943	131925	0.009	0.976	14.6319	15:0 iso	45.87	ECL deviates 0.000	Reference 0.004
2.4229	3023	0.009	0.973	14.7246	15:0 anteiso	1.05	ECL deviates 0.000	Reference 0.003
2.4695	4855	0.009	0.968	14.8758	15:1 w6c	1.67	ECL deviates 0.001	
2.5077	3172	0.011	----	14.9996	15:0	----	ECL deviates 0.000	
2.6621	889	0.013	0.951	15.4854	16:1 iso H	0.30	ECL deviates 0.003	
2.7095	515	0.010	0.947	15.6347	16:0 iso	0.17	ECL deviates 0.002	Reference 0.003
2.7747	73410	0.009	0.941	15.8394	Sum In Feature 3	24.62	ECL deviates -0.001	16:1 w7c/16:1 w6c
2.7870	18253	0.008	0.940	15.8782	Sum In Feature 3	6.12	ECL deviates 0.003	16:1 w6c/16:1 w7c
2.8020	7582	0.009	0.939	15.9254	16:1 w5c	2.54	ECL deviates -0.003	
2.8258	3839	0.009	0.937	16.0001	16:0	1.28	ECL deviates 0.000	Reference 0.001
2.8776	3931	0.009	0.933	16.1629	15:0 iso 3OH	1.31	ECL deviates 0.001	
2.9673	29852	0.009	0.927	16.4442	Sum In Feature 9	9.86	ECL deviates -0.003	17:1 iso w9c
2.9899	4090	0.009	0.926	16.5152	Sum In Feature 4	1.35	ECL deviates 0.003	17:1 anteiso B/iso I
3.0025	612	0.008	0.925	16.5547	17:1 anteiso w9c	0.20	ECL deviates 0.003	
3.0285	1193	0.009	0.923	16.6362	17:0 iso	0.39	ECL deviates -0.001	Reference 0.000
3.0859	1244	0.011	0.919	16.8165	17:1 w8c	0.41	ECL deviates 0.002	
3.1076	541	0.011	0.918	16.8844	17:1 w6c	0.18	ECL deviates 0.003	
3.4846	2036	0.018	0.899	18.0795	18:1 w7c 11-methyl	0.65	ECL deviates -0.006	

ECL Deviation: 0.002 Reference ECL Shift: 0.007 Number Reference Peaks: 7
Total Response: 298885 Total Named: 293510
Percent Named: 98.20% Total Amount: 280644

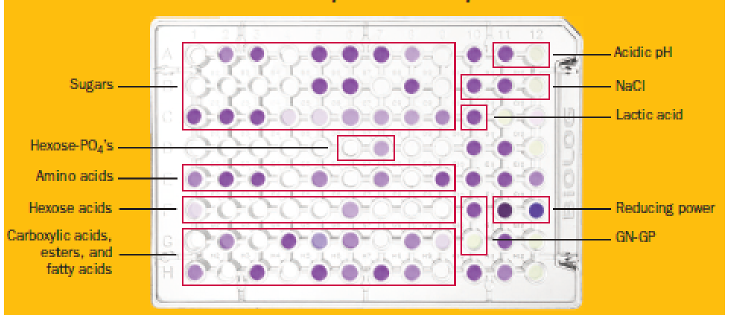
Matches:

Library	Sim Index	Entry Name
ITSA1 1.10	0.363	Flavobacterium-mizutaii

Anatomy of a GEN III identification.

The new GEN III redox chemistry is applicable to an unprecedented range of both gram negative and gram positive bacteria. As shown below, GEN III dissects and analyzes the ability of the cell to metabolize all major classes of biochemicals, in addition to determining other important physiological properties such as pH, salt, and lactic acid tolerance, reducing power, and chemical sensitivity. Identifications can be performed manually, or with all Biolog instruments including the semi-automated MicroStation™ and the automated OmniLog®.

ID = *Stenotrophomonas maltophilia*



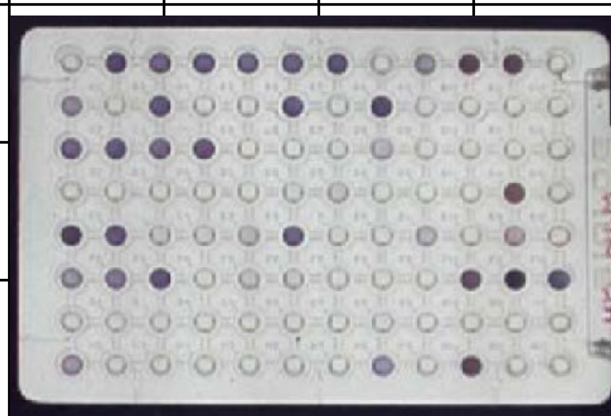
71 Carbon Source *plus* 23 Chemical Sensitivity Assays

BIOLOG, Inc.
21124 Cabot Blvd
Hayward, CA 94545 USA
(510) 785-2564 · www.biolog.com

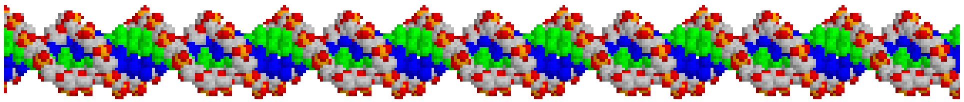
BIOLOG

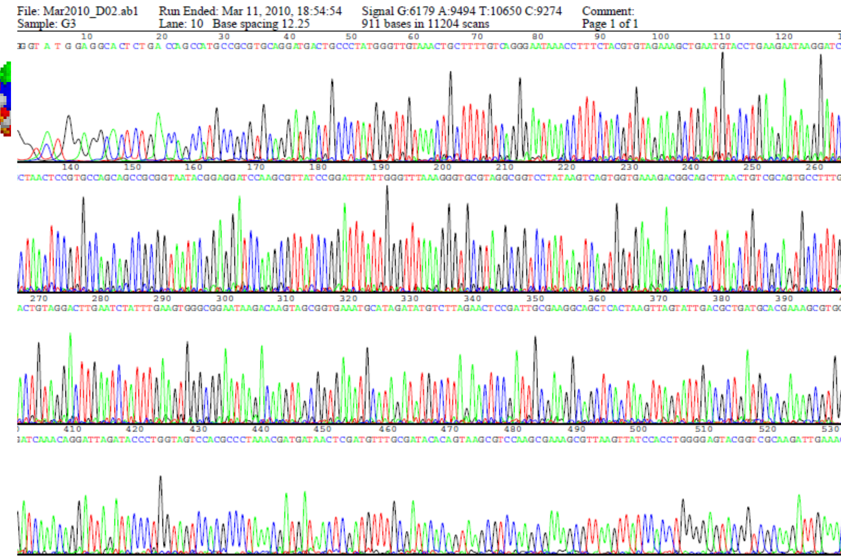
Biolog GenIII Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	negative control	**Dextrin** amylose glu-α1-4, α1-6 poly	**D-maltose** glu-α1-4-glu	**D-trehalose** glu-α1-α1-glu	**D-cellobiose** glu-β1-4-glu	**gentiobiose** glu-β1-6-glu	**Sucrose** glu-α1-β2-fru	**D-turanose** glu-α1-α3-fru	**Stachyose** gal(α1→6) gal(α1→6) glc(α1↔2β) fru	positive control	pH 6	pH 5
B	**D-raffinose** gal-α1-6-glu-α1-β2-fru	**α-D-lactose** gal-β1-4-glu (α)	**D-melibiose** gal-α1-6-glu	β-methyl-D-glucoside	**D-salicin** aspirin-β1-glu	N-acetyl-D-glucos-amine	N-acetyl-β-D-mannos-amine	N-acetyl-D-galactos-amine	N-acetyl neuraminic acid	1% NaCl	4% NaCl	8% NaCl
C	α-D-glucose	D-mannose	D-fructose	D-galactose	3-methyl glucose	D-fucose	L-fucose	L-rhamnose	inosine	1% Na-lactate	fusidic acid	D-serine
D	D-sorbitol	D-mannitol	D-arabitol	myo-inositol	glycerol	D-glucose-6-PO4	D-fructose-6-PO4	D-aspartic acid	D-serine	Troleando-mycin	rifamycin SV	minocycline
E	gelatin	glycyl-L-proline	L-alanine	L-arginine	L-aspartic acid	L-glutamic acid	L-histidine	L-pyro-glutamic acid	L-serine	lincomycin	guanidine HCl	niaproof 4
F	pectin	D-galact-uronic acid	L-galact-uronic acid lactone					quinic acid	D-saccharic acid	vancomycin	tetrazolium violet	tetrazolium blue
G	p-hydroxy-phenyl-acetic acid	methyl pyruvate	D-lactic acid methyl ester					L-malic acid	bromo-succinic acid	nalidixic acid	LiCl	K-tellurite
H	tween-40	γ-amino-butyric acid	α-hydroxy-butyric acid					acetic acid	formic acid	aztreonam	Na-butyrate	Na bromate



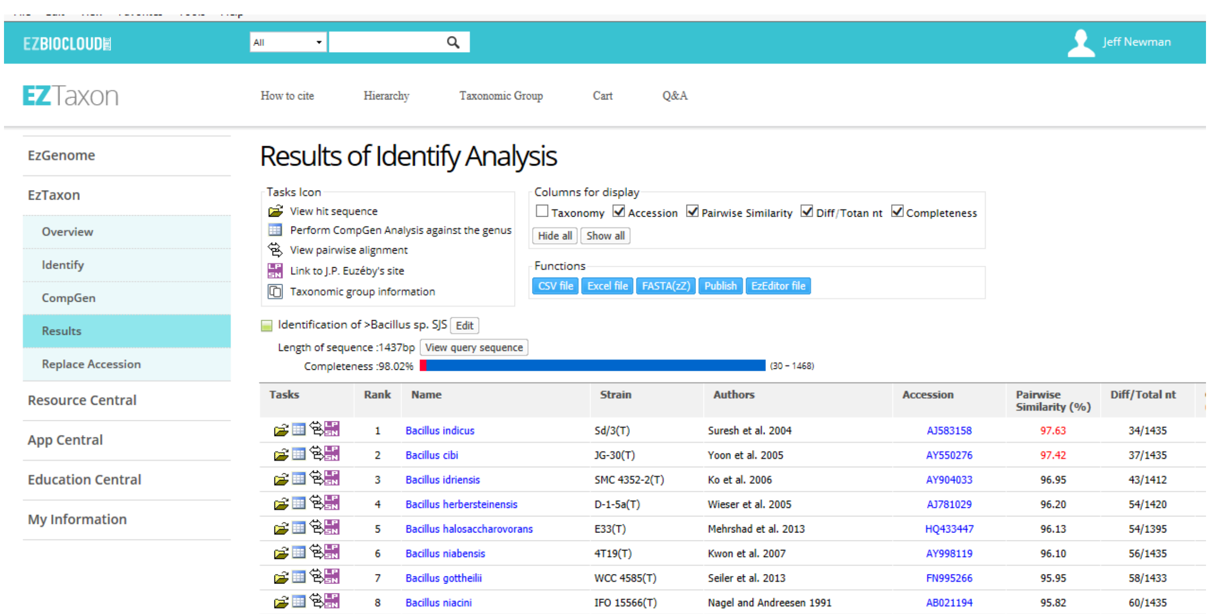
The LycoMicro Unknown Microbe Lab

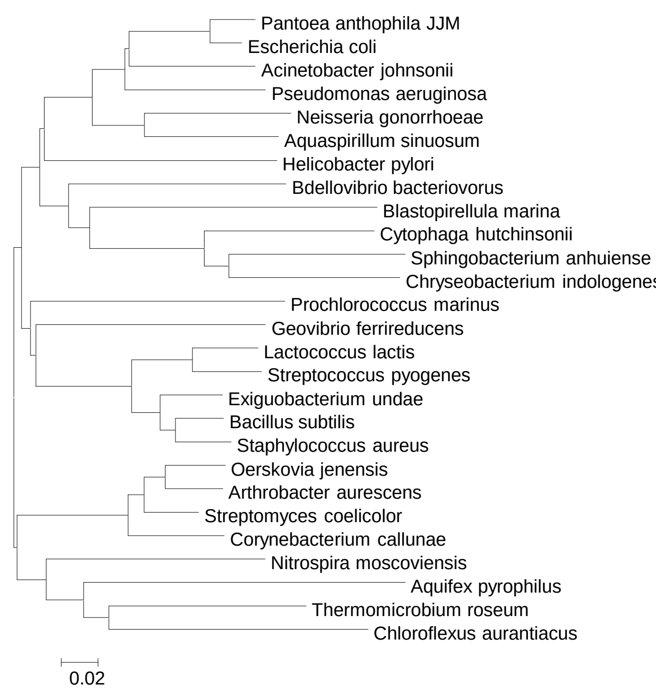




Week 8 – Analyze DNA sequence @ http://www.ezbiocloud.net/eztaxon ,

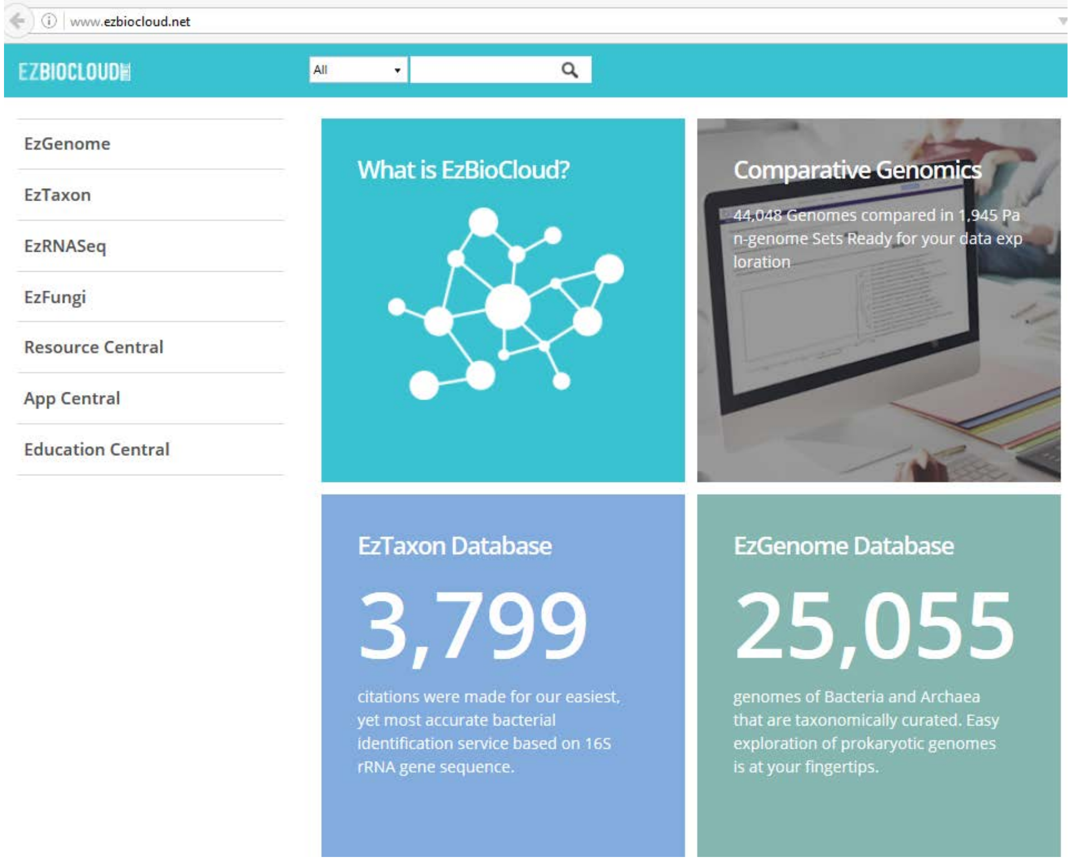
- Construct Phylogenetic Tree w/MEGA,
- Literature Research (IJSEM)



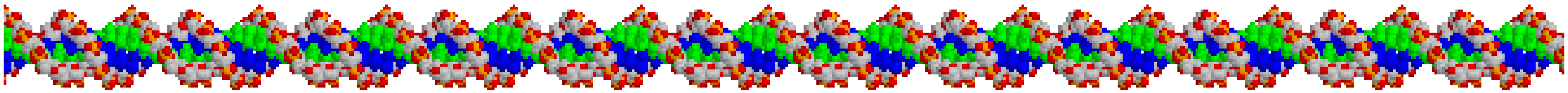


EZ-Taxon

- http://www.ezbiocloud.net/eztaxon
- Database contains type strains = official representatives of a species
  - Good pairwise scores
  - “CompGen” compares to all members of Genus, can easily download and format sequences for trees



EZ-Taxon



What is threshold for positive ID?

EZBIOCLOUD All Jeff Newman Logout

EzTaxon How to cite Hierarchy Taxonomic Group Cart Q&A

- EzGenome
- EzTaxon
  - Overview
  - Identify
  - CompGen
  - Results
  - Assemble
  - Replace Accession
- EzRNASeq
- EzFungi
- Resource Central
- App Central
- Education Central
- My Information

Results of Identify Analysis

Tasks Icon
- View hit sequence
- Perform CompGen Analysis against the genus
- View pairwise alignment
- Link to J.P. Euzéby's site
- Taxonomic group information

Columns for display
☐ Taxonomy ☑ Accession ☑ Pairwise Similarity ☑ Diff/Totan nt ☑ Completeness
Hide all Show all

Functions
CSV file Excel file FASTA(zZ) Publish EzEditor file

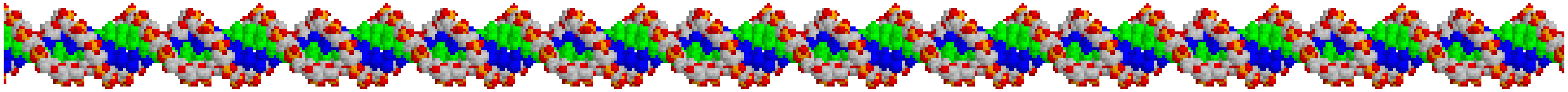
Identification of Flavobacterium sp. NLM] Edit

Length of sequence :1509bp View query sequence

Completeness :100% (1 – 1510)

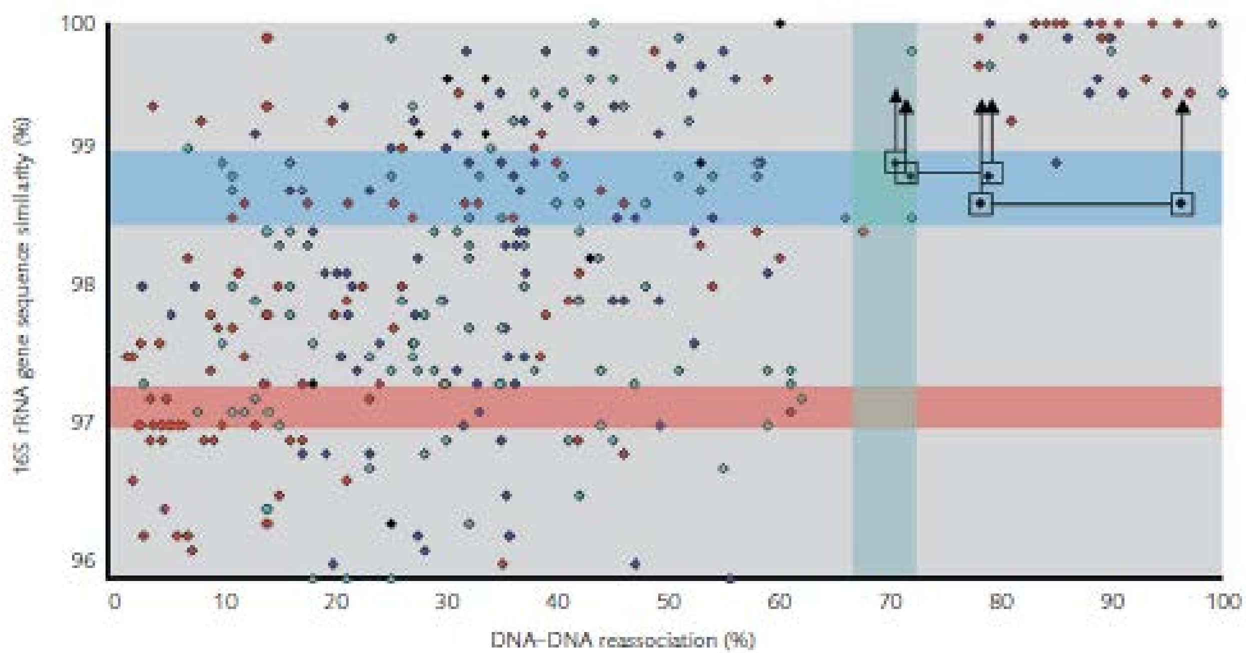
Tasks	Rank	Name	Strain	Authors	Accession	Pairwise Similarity (%)	Diff/Total nt
	1	Flavobacterium resistens	BD-b365(T)	Ryu et al. 2008	EF575563	97.69	34/1472
	2	Flavobacterium aquidurense	WB-1.1.56(T)	Cousin et al. 2007	AM177392	97.40	38/1460
	3	Flavobacterium tructae	435-08(T)	Zamora et al. 2014	HE612100	97.39	38/1458
	4	Flavobacterium reichenbachii	LMG 25512(T)	Ali et al. 2009	JPRL01000002	97.35	39/1472
	5	Flavobacterium ginsenosidimutans	THG 01(T)	Yang et al. 2011	GU138377	97.34	37/1392
	6	Flavobacterium chilense	LM-09-Fp(T)	Kämpfer et al. 2012	FR774915	97.19	39/1388
	7	Flavobacterium spartansii	T16(T)	Loch and Faisal 2014	JX287799	97.18	39/1383
	8	Flavobacterium araucananum	LM-19-Fp(T)	Kämpfer et al. 2012	FR774916	97.13	40/1392
	9	Flavobacterium chungangense	LMG 26729(T)	Kim et al. 2009	JASY01000008	97.08	43/1472
	10	Flavobacterium piscis	412R-09(T)	Zamora et al. 2014	HE612101	97.05	43/1459

How are bacterial species defined?



- The Gold Standard
  - Less than 70% DNA-DNA hybridization (green line)
- % Identity with 16S rDNA sequence
  - 97% (red line) vs. 98.5% (blue line)… but only 1 gene

- Fig from Stackebrandt & Ebers, 2006, Microbiol.Today 33:152

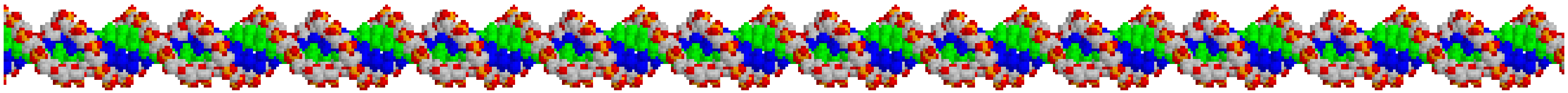


Systematics and Taxonomy

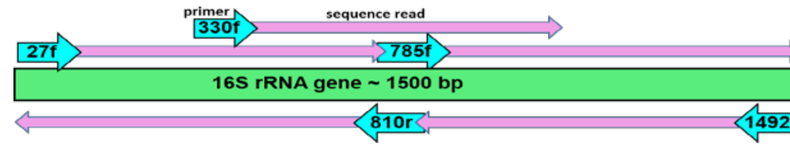
- Official definition of prokaryotic species is 70% DNA-DNA hybridization (DDH) – difficult to physically measure
- ~70% DDH = ~98.7% identical 16S rRNA sequence
  = ~95% average nucleotide identity (ANI)
  = ~95% amino acid identity (AAI)

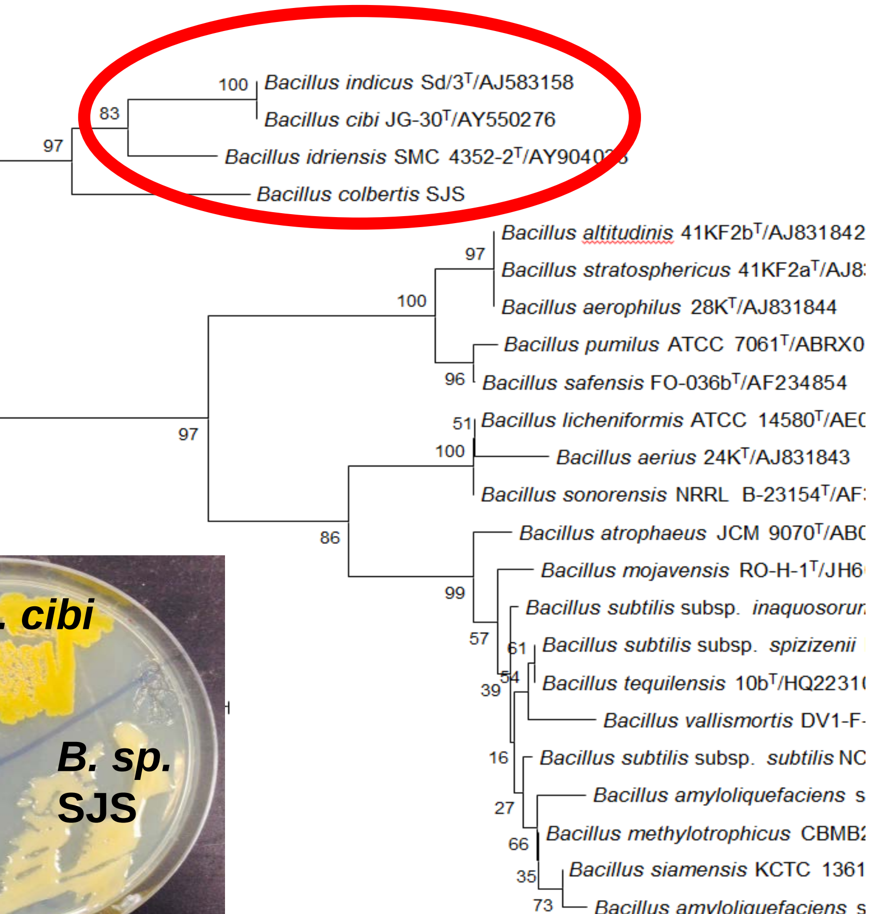
- New species, genera, families should be **compared to closest relatives** & described in the International Journal of Systematic and Evolutionary Microbiology (IJSEM)
  – **Type species, Type strain** is “official” representative of group
  – Deposit in 2 culture collections
    ATCC, DSMZ, JCM, BCCM/LMG, CCUG, KCTC
  – Sequences (at least 16S rRNA) deposited in GenBank

Bio447 - Research Methods



- Complete & deposit 16S sequence
- Determine reference organisms from phylogenetic tree
- Sequence & compare genome(s)
- Obtain reference organisms
- Repeat experiments in parallel to determine differences and similarities
- Prepare poster for ASM
- Write a paper for IJSEM





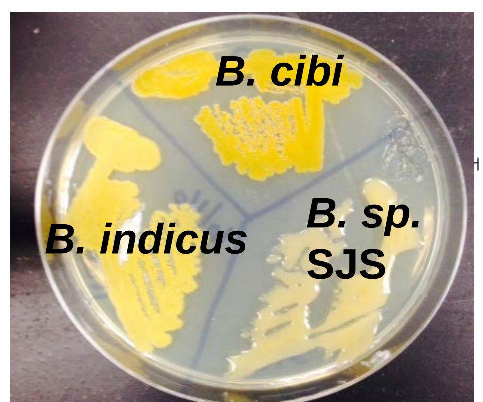
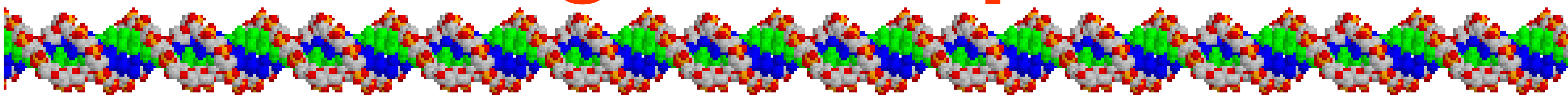


Table 1. Fatty acid composition (%) of *Bacillus sp* SJS, *B. indicus* LMG 22858^T, and *B. cibi* DSM 16189^T

Fatty Acids

Fatty Acid	1	2	3
iso-$C_{14:0}$	**17.3**	**7.2**	**5.9**
$C_{14:0}$	2.1	2.8	2.1
iso-$C_{15:0}$	**20.3**	**46.4**	**48.9**
anteiso-$C_{15:0}$	**30.0**	**15.7**	**14.9**
$C_{16:1}$ ω7c alcohol	4.5	4.6	4.9
iso-$C_{16:0}$	8.7	3.4	3.7
$C_{16:1}$ ω11c	2.2	5.6	5.7
$C_{16:0}$	8.2	4.4	3.2
iso-$C_{17:1}$ ω10c	1.93	2.5	3.3
Summed in Feature 4	0.3	2.0	2.2
iso-$C_{17:0}$	1.9	1.7	1.6
anteiso-$C_{17:0}$	3.9	2.3	2.4

Biolog GenIII plates



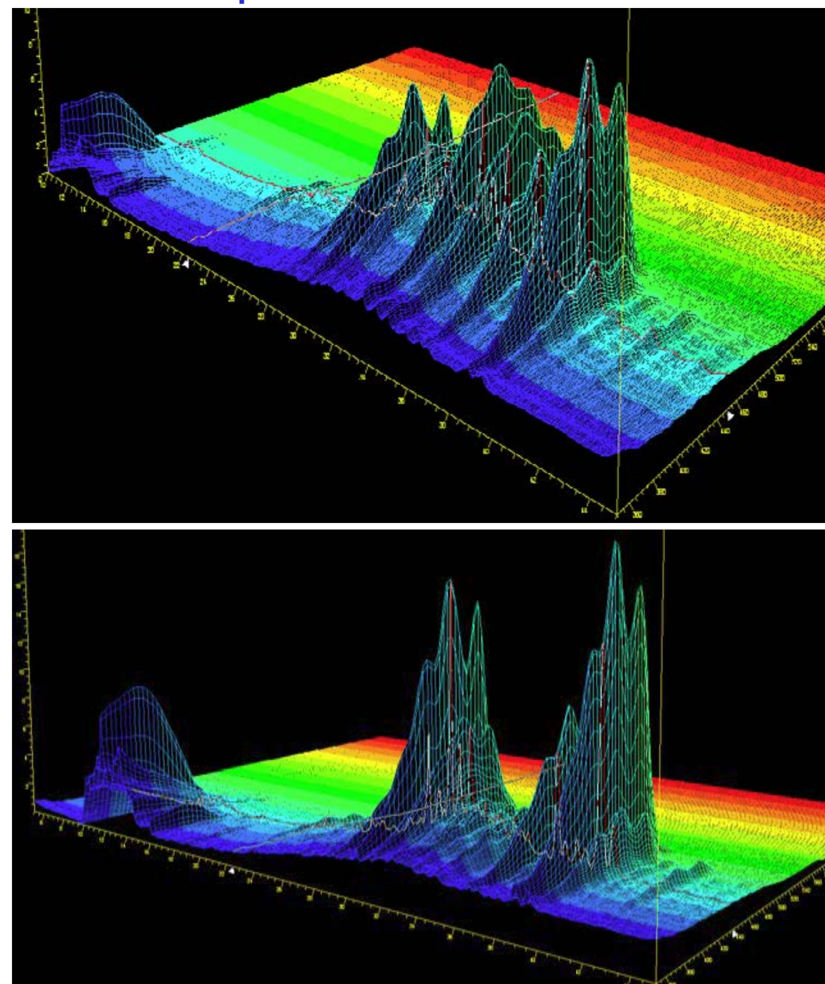
Name	Bacillus colbertis	Bacillus indicus	Bacillus cibi	Name	Bacillus colbertis	Bacillus indicus	Bacillus cibi	Name	Bacillus colbertis	Bacillus indicus	Bacillus cibi	Name	Bacillus colbertis	Bacillus indicus	Bacillus cibi
neg control	24	35	24	α-D-glucose	27	31	55	gelatin	60	100	99	p-hydroxy-phenylacetic acid	12	6	9
dextrin	78	98	100	D-mannose	6	45	1	glycyl-L-proline	3	50	60	methyl pyruvate	11	93	91
D-maltose	17	48	45	D-fructose	15	77	77	L-alanine	20	93	97	D-lactic acid methyl ester	5	63	77
D-trehalose	17	32	43	D-galactose	9	27	62	L-arginine	8	51	41	L-lactic acid	4	97	90
D-cellobiose	8	26	22	3-methyl glucose	5	2	4	L-aspartic acid	6	63	60	citric acid	3	18	27
gentiobiose	22	77	71	D-fucose	3	12	8	L-glutamic acid	9	84	88	a-keto-glutaric acid	14	96	95
sucrose	17	8	16	L-fucose	5	5	6	L-histidine	8	85	95	D-malic acid	3	11	27
D-turanose	39	95	98	L-rhamnose	4	3	2	L-pyroglutamic acid	18	83	73	L-malic acid	13	64	97
stachyose	21	59	73	inosine	8	14	18	L-serine	36	85	88	bromo-succinic acid	4	0	11
pos control	94	94	95	1% Na-lactate	98	84	81	lincomycin	7	6	6	nalidixic acid	12	36	13
pH 6	97	92	94	fusidic acid	8	6	6	guanidine HCl	7	6	6	LiCl	84	91	93
pH 5	23	6	6	D-serine	8	6	6	niaproof 4	10	6	6	K-tellurite	98	93	95
D-raffinose	17	45	65	D-sorbitol	12	12	14	pectin	55	97	99	tween-40	41	99	98
α-D-lactose	8	12	14	D-mannitol	11	5	14	D-galacturonic acid	11	25	24	g-amino-butyric acid	3	1	1
D-melibiose	8	59	67	D-arabitol	3	2	2	L-galacturonic acid lactone	4	6	5	a-hydroxy-butyric acid	3	11	7
β-methyl-D-glucoside	12	37	46	myo-inositol	4	1	2	D-gluconic acid	9	98	97	b-hydroxy-D,L-butyric acid	45	99	99
D-salicin	11	62	72	glycerol	10	63	77	D-glucuronic acid	5	75	68	a-keto-butyric acid	3	3	3
N-acetyl-D-glucosamine	12	72	85	D-glucose-6-PO4	5	22	21	glucuronamide	8	40	12	acetoacetic acid	17	84	88
N-acetyl-β-D-mannosamine	14	21	27	D-fructose-6-PO4	10	41	49	mucic acid	4	14	24	propionic acid	4	5	12
N-acetyl-D-galactosamine	4	4	5	D-aspartic acid	4	2	1	quinic acid	5	43	36	acetic acid	11	91	91
N-acetyl neuraminic acid	6	3	6	D-serine	3	0	1	D-saccharic acid	5	35	20	formic acid	98	5	10
1% NaCl	99	92	97	troleandomycin	7	6	6	vancomycin	6	6	6	aztreonam	9	90	93
4% NaCl	93	91	93	rifamycin SV	9	6	39	tetrazolium violet	25	16	16	Na-butyrate	87	87	46
8% NaCl	74	92	93	minocycline	12	6	7	tetrazolium blue	19	11	10	Na bromate	37	9	13

Pigment Comparison

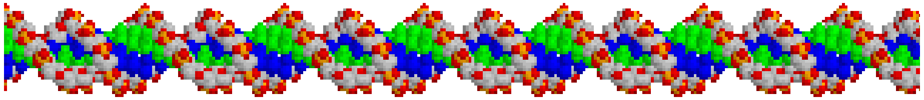
Pigmentation after 72hrs growth on TSA medium at 30^{o}C.

HPLC pigment results:

B. cibi top, *B. indicus* bottom

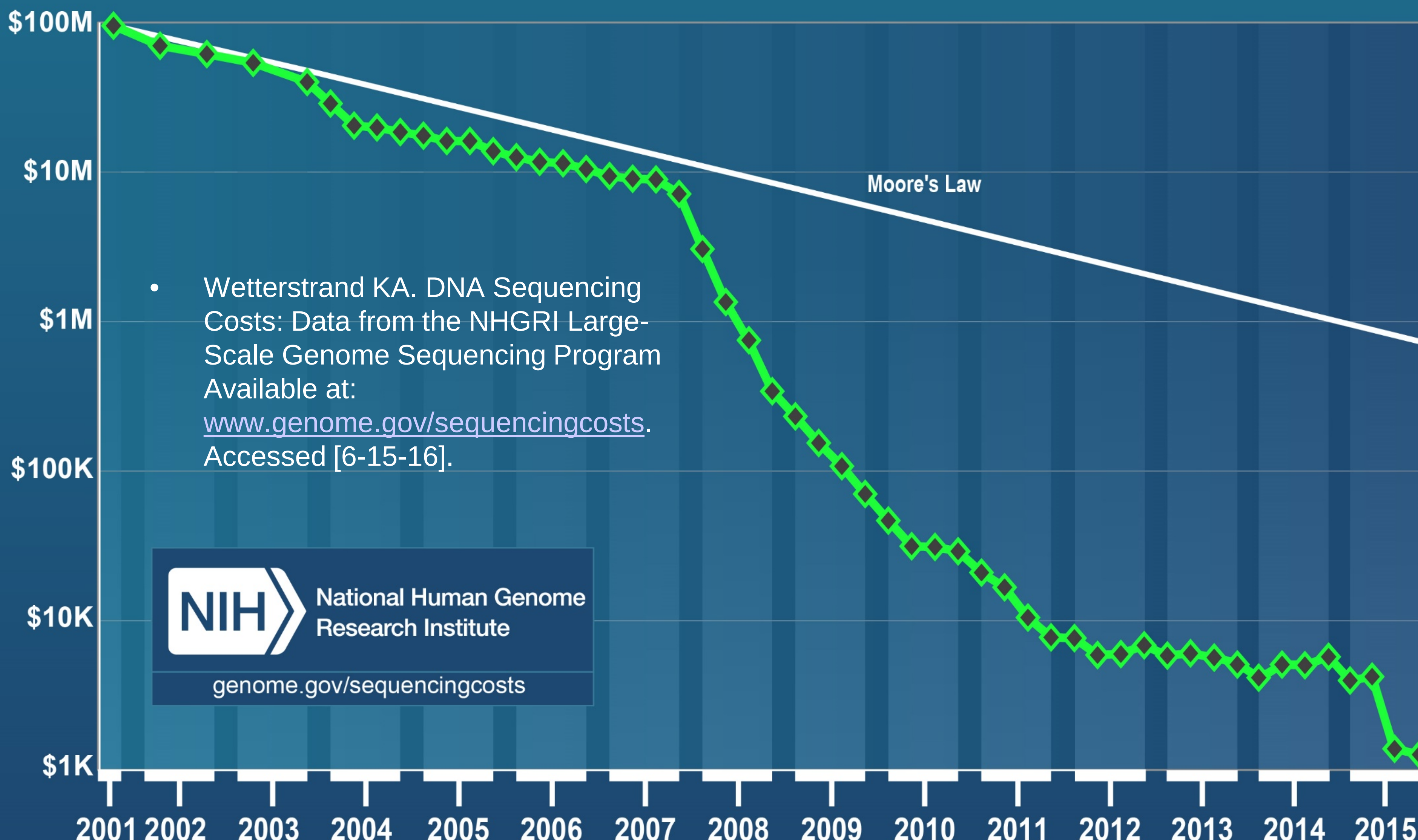


The Journey



- Starting point: CURE - Micro UK's from freshwater creeks, 16S seq → novel species
- Compare phenotypes to closest type strains
  - Literature → family/genus specific tests
- Minimal Standards
- **Best Practices: DDH→MLST→Genomes**
- Calculate Phylogenomic Metrics (GGDC, ANI, AAI, ROSA)
- RAST/SEED viewer for genome analysis, correlation with phenotypes
- Simple web/excel tools use RAST output to:
  - Calculate AAI, BBH, ROSA
  - ID unique and shared genes
  - Create Venn Diagram
- Publish new genera & species!

Cost per Genome



- Wetterstrand KA. DNA Sequencing Costs: Data from the NHGRI Large-Scale Genome Sequencing Program Available at: www.genome.gov/sequencingcosts. Accessed [6-15-16].

HHMI

GCAT→ GCAT-SEEK

NSF

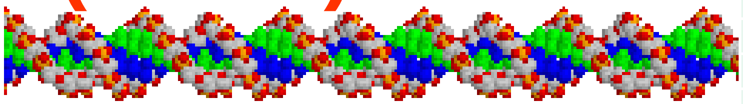
- Genome Consortium for Active Teaching (GCAT) founded in 2000 to bring Genomics (Microarrays) to the undergraduate curriculum.
- Multiple HHMI & NSF funded workshops

- GCAT-SEEKquence “spin-off” to bring NextGen sequencing to the undergraduate curriculum.
- 3 genomes (Ion Torrent & 454 as part of pilot)
- NSF Research Collaboration Network, Juniata’s HHMI Genomics Leadership Initiative

Genome Consortium for Active Teaching

SEEKquence

Shared MiSeq (2x300) Runs



- NextGen Instruments generate more data than most UG faculty can use or afford.

- November 2013 – 27 bacteria @$200 each (including *Flavobacterium aquatile)*

- April, 2014 – Opened to Microedu Listserv → 35 Bacteria and Phage from 16 institutions @$190/sample

- October 2014 – 30 phage, viruses and bacteria @$175/sample.

Sample	Reads est.	Bases est.
GSF665-1-E_coli-C06b	217,320	130,391,966
GSF665-2-Chryseobacterium-LO	1,317,872	790,723,170
GSF665-3-Linfield-KH	809,893	485,935,870
GSF665-4-Linfield-NH	301,171	180,702,758
GSF665-5-Exiguobacterium	794,482	476,689,384
GSF665-6-Plesiomonas_shigelloides	656,143	393,685,659
GSF665-7-Halosimplex_carlsbadense	595,655	357,393,201
GSF665-8-Phage_Eapen	573,447	344,068,354
GSF665-9-Phage_Aspire	170,895	102,536,927
GSF665-10-strain_3572	593,179	355,907,159
GSF665-11-Gracilibacillus_dipsosauri	986,925	592,154,880
GSF665-12-Serratia_S12	827,533	496,519,794
GSF665-13-Rhodococcus_T1Sofl-14	297,153	178,292,067
GSF665-14-Janthinobacterium-BJB1	823,488	494,092,592
GSF665-15-Janthinobacterium-BJB349	883,287	529,972,260
GSF665-16-Janthinobacterium-BJB304	1,098,516	659,109,346
GSF665-17-Janthinobacterium-BJB317	549,616	329,769,324
GSF665-18-Iodobacter-BJB302	206,973	124,183,611
GSF665-19-Asaia_bogorensis	1,096,204	657,722,373
GSF665-20-Asaia_siamensis	820,818	492,490,968
GSF665-21-Asaia_astilbes	783,447	470,068,239
GSF665-22-Asaia_platycodi	808,325	484,994,710
GSF665-23-Asaia_krungthepensis	1,152,811	691,686,698
GSF665-24-Asaia_prunellae	1,035,414	621,248,288
GSF665-27-Serratia -DL	129,258	77,554,903
GSF665-28-Phage-KitKat	53,773	32,263,632
GSF665-29-Cyanobacterium-RC610	909,265	545,559,194
GSF665-30-Serratia_marcescens-RH	307,886	184,731,584
GSF665-31-Bacillus_cibi	693,101	415,860,714
GSF665-32-Pedobacter-BMA	1,200,365	720,218,713
GSF665-33-Flavobacterium-KMS	185,975	111,585,274
GSF665-34-Flavobacterium_hibernum	1,432,517	859,510,422
GSF665-36-Flavobacterium_hydatis	744,893	446,935,512
GSF665-39-Kaistella_koreensis	1,238,892	743,334,928
GSF665-40-Kaistella_haifense	1,067,969	640,781,490
Total	**25,364,460**	**15,218,675,963**
Average	**724,699**	**434,819,313**

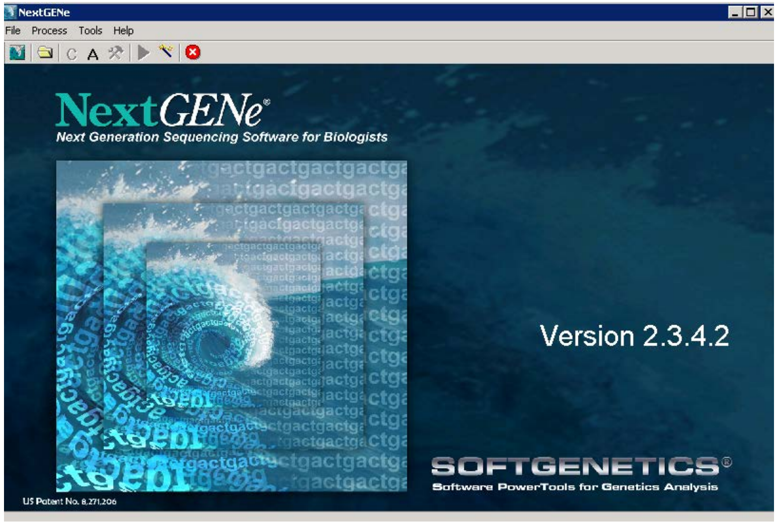
Assembly statistics

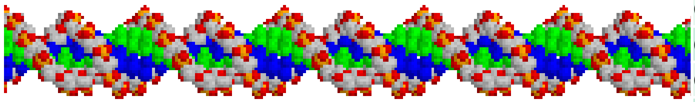
[SoftGenetics Assembler: Assembly Results Statistics Report]

- **Total Reads Number: 2056329**
- Matched Reads Number: 1983986
- Unmatched Reads Number: 72343
- **Assembled Sequences Number: 61**
- Average Sequence Length: 57497
- Minimum Sequence Length: 158
- Maximum Sequence Length: 641985
- **N50 Length: 366076**

[Final Contig Merge Results Statistics Report]

- **Final Contig Merge Sequences Number: 13**
- Final Contig Merge Average Sequence Length: 269063
- Final Contig Merge Minimum Sequence Length: 173
- Final Contig Merge Maximum Sequence Length: 856388
- **Final Contig Merge N50 Length: 586767**
- Matched Reads Count: 1977550
- Number of Matched Bases: 562514128
- **Average Read Length: 285**
- **Average Coverage: 161**
- **Reference Length: 3507364**





Genomes deposited in GenBank

species	strain	WGS	genome size	contigs
Bacillus cibi	DSM 16189	JNVC02	4,072,175	24
Bacillus indicus	LMG 22858	JGVU02	4,129,127	22
Bacillus sp SJS (colbertis)	SJS	JAQV02	4,148,893	36
Chryseobacterium angstadtii	KM	LFND01	5,202,773	11
Chryseobacterium antarcticum	LMG 24720	JPEP01	3,123,663	4
Chryseobacterium aquaticum	KCTC 12483	LLYZ01	3,813,178	21
Chryseobacterium formosense	LMG 24722	JPRP01	4,364,663	10
Chryseobacterium greenlandense	UMB34	LMAI01	4,036,521	21
Chryseobacterium hispalense	DSM 25574	JARQ01	4,363,762	27
Chryseobacterium jeonii	DSM 17048	JSYL01	3,261,282	35
Chryseobacterium luteum	DSM 18605	JPRO01	4,718,546	49
Chryseobacterium piperi	CTM	JPRJ01	4,340,594	89
Chryseobacterium soli	DSM 19298	JPRH01	4,754,478	26
Chryseobacterium solincola	DSM 22468	JSYK01	2,345,236	14
Chryseobacterium sp BLS98	BLS98	LFNF01	4,412,600	8
Chryseobacterium sp FH2	FH2	LFNE01	3,988,758	24
Chryseobacterium sp. JM1	JM1	JPRN01	5,256,156	31
Chryseobacterium vrystaatense	LMG 22846	JPRI01	5,487,842	18
Epilithonimonas diehli FH1	FH1	JPLZ01	3,975,884	12
Epilithonimonas lactis	LMG 24401	JPLY01	4,236,390	22
Flavobacterium aquatile	LMG 4008	JRHH01	3,490,856	7
Flavobacterium chilense	LMG 26360	LSYT01	5,924,510	21
Flavobacterium chungangense	LMG 26729	JASY01	5,591,549	45
Flavobacterium hibernum	DSM 12611	JPRK01	5,283,672	30
Flavobacterium hydatis	DSM 2063	JPRM01	5,877,671	99
Flavobacterium reichenbachii	LMG 25512	JPRL01	5,501,401	12
Flavobacterium sp AED (franzi)	AED	JSYM01	3,925,153	15
Flavobacterium sp JRM	JRM	JSYO01	5,380,719	169
Flavobacterium sp KJJ	KJJ	JARP01	4,603,121	10
Flavobacterium sp KMS	KMS	JSYP01	5,597,129	58
Flavobacterium sp VMW	VMW	LHVT01	5,627,095	59
Flavobacterium sp. ABG (douthatii)	ABG	LEKS01	5,270,010	89
Flavobacterium succinicans	LMG 10402	JATV01	3,652,806	48
Kaistella koreensis	CCUG	LFNG01	3,154,203	64
Pedobacter borealis	DSM 19626	JAUG01	5,544,917	216
Pedobacter ginsenosidimutans	KACC 14530	LMZQ01	6,517,553	86
Pedobacter kyungheensis	KACC 16221	JSYN01	6,358,642	67
Pedobacter sp 20-19	R20-19	JCKI01	5,006,523	15
Pedobacter sp. BMA	BMA	LECU01	5,040,634	20

95% ANI ~ 70% DDH ~ 69% Conserved (>90%)

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DNA–DNA hybridization values and their relationship to whole-genome sequence similarities

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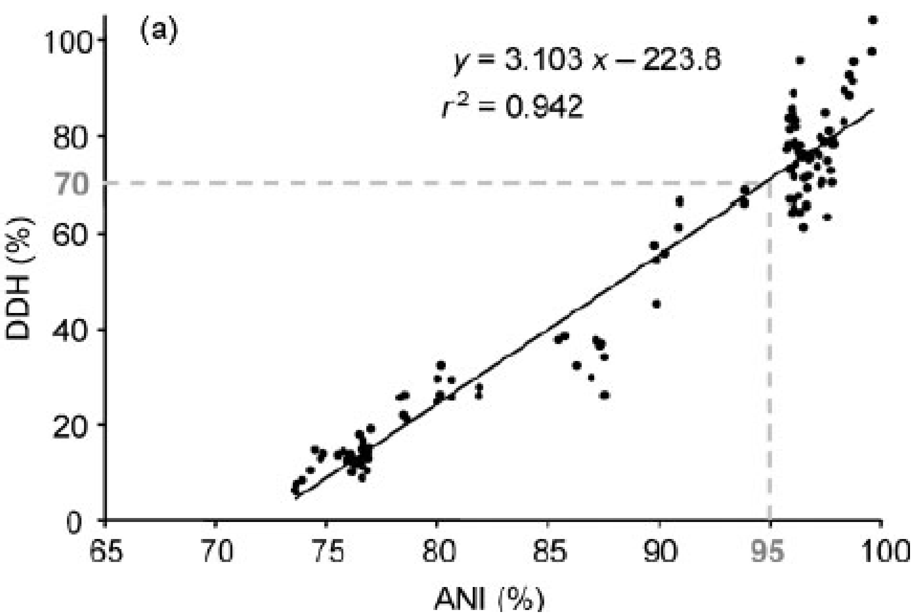
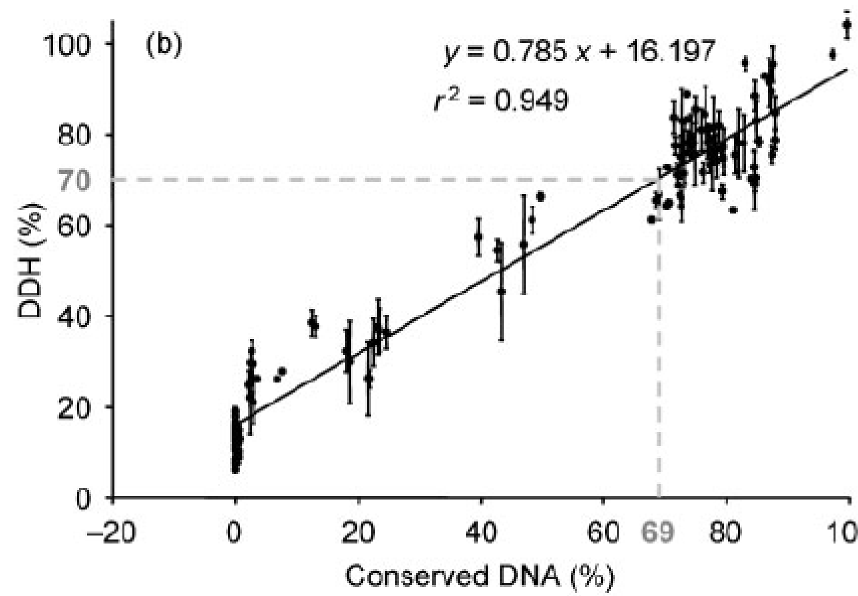
DNA–DNA hybridization (DDH) values have been used by bacterial taxonomists since the 1960s to determine relatedness between strains and are still the most important criterion in the delineation of bacterial species. Since the extent of hybridization between a pair of strains is ultimately governed





Shifting the genomic gold standard for the prokaryotic species definition

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Edited by James M. Tiedje, Center for Microbial Ecology, East Lansing, MI, and approved September 16, 2009 (received for review June 11, 2009)

DNA-DNA hybridization (DDH) has been used for nearly 50 years as the gold standard for prokaryotic species circumscriptions at the genomic level. It has been the only taxonomic method that offered a numerical and relatively stable species boundary, and its use has had a paramount influence on how the current classification has been constructed. However, now, in the era of genomics, DDH appears to be an outdated method for classification that needs to be substituted. The average nucleotide identity (ANI) between two genomes seems the most promising method since it mirrors DDH closely. Here we examine the work package JSpecies as a user-friendly, biologist-oriented interface to calculate ANI and the correlation of the tetranucleotide signatures between pairwise genomic comparisons. The results agreed with the use of ANI to substitute DDH, with a narrowed boundary that could be set at ≈95–96%. In addition, the JSpecies package implemented the tetranucleotide signature correlation index, an alignment-free parameter that generally correlates with ANI and that can be of help in deciding when a given pair of organisms should be classified in the same species. Moreover, for taxonomic purposes, the analyses can be produced by simply randomly sequencing at least 20% of the genome of the query strains rather than obtaining their full sequence.

average nucleotide identity | DNA-DNA hybridization | genome-based taxonomy | tetranucleotide regression

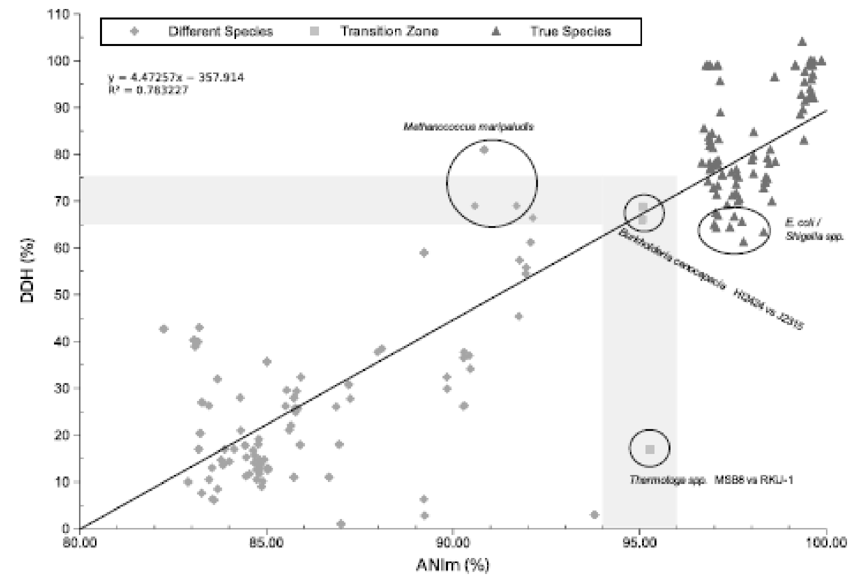

Fig. 2. Plotted values of DDH versus ANIm. Triangles show values that correspond to what taxonomists consider as "true" species according to the DDH values traditionally applied and that have previously been classified. Squares indicate values that appear to be in the transition zone.

www.pnas.org/cgi/doi/10.1073/pnas.0906412106

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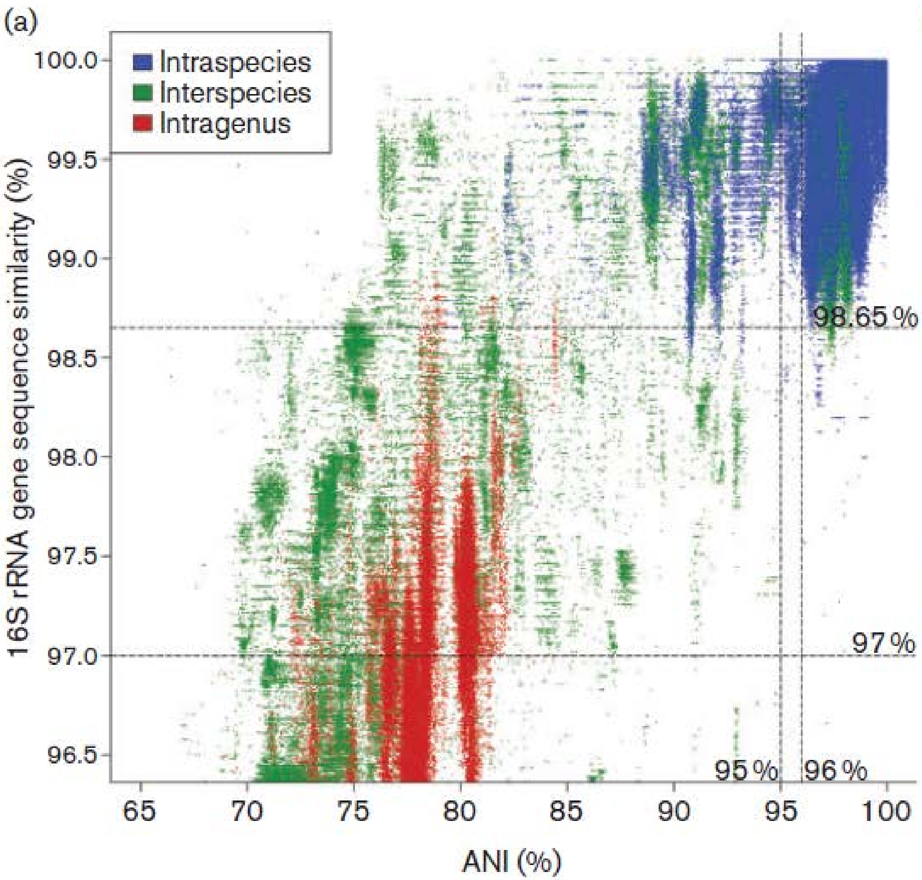
Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes

Mincheol Kim,[1] Hyun-Seok Oh,[2] Sang-Cheol Park[2] and Jongsik Chun[1,2]

(a)



(b)

16S rRNA gene sequence similarity (%): 100.0, 99.0, 98.9, 98.8, 98.7, 98.65, 98.6, 98.5, 98.4, 98.3, 98.2, 98.1, 98.0, 97.0, 96.0, 95.0

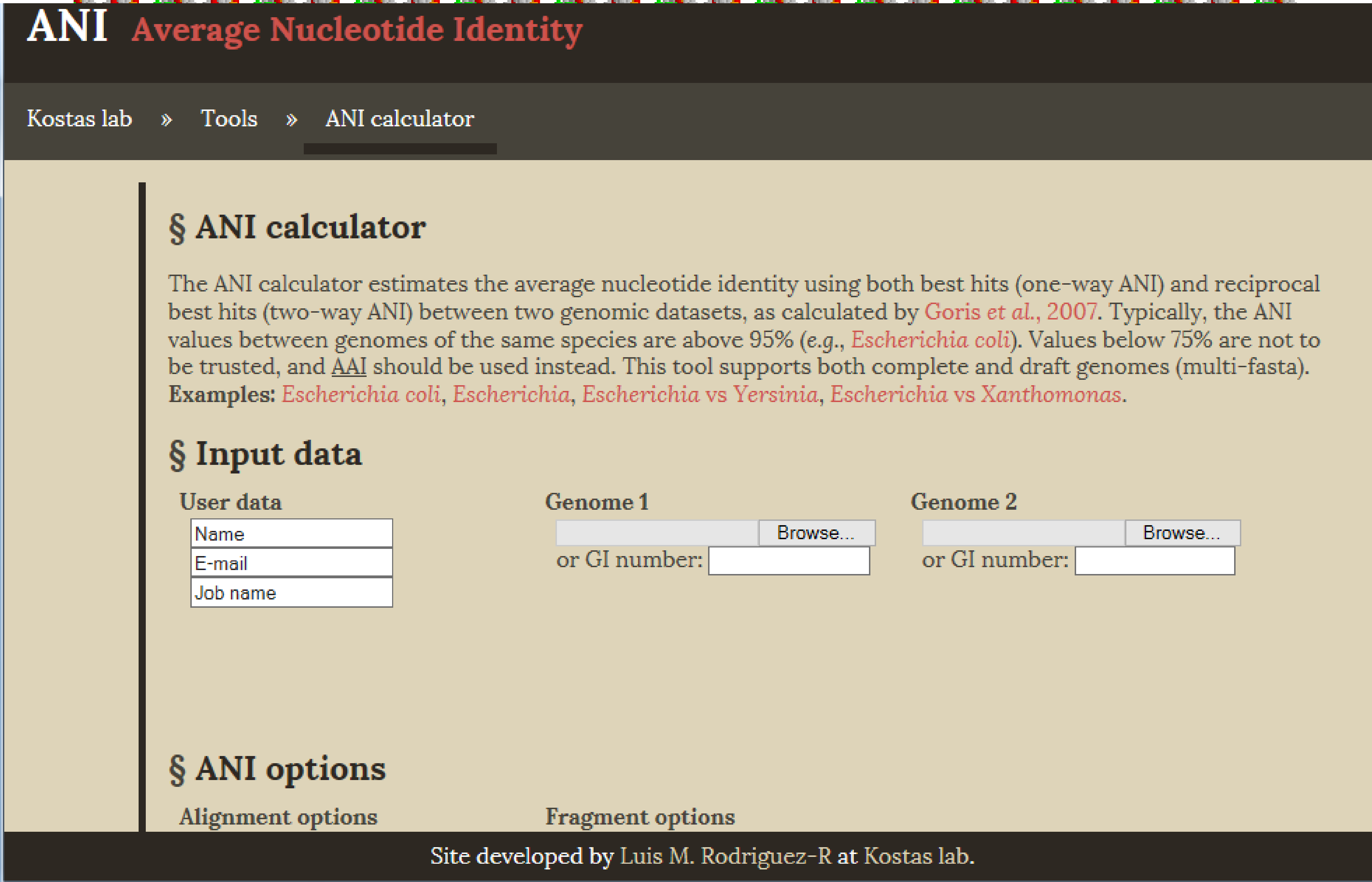
3438	9394	4373	4856	7828	5028	58692	75205	134067	93556
206	185	180	12	2	11	1658	1440	619	12
267	276	138	9	4	18	970	1077	369	7
165	177	71	3	1	12	547	739	235	3
153	109	55	6	2	5	326	385	146	1
109	32	40	16	1	1	126	263	89	0
90	23	31	6	0	1	66	216	31	4
77	13	15	24	2	3	27	116	16	1
80	7	14	46	0	1	10	70	3	0
45	4	6	10	0	1	10	38	3	0
36	10	8	7	1	2	11	14	1	0
18	4	6	3	1	3	39	6	0	0
8	6	9	4	1	7	7	1	0	0
12	33	10	5	0	5	27	0	1	0
14	167	8	5	4	3	47	0	0	0
1	286	15	5	2	1	9	0	0	1
0	179	24	20	1	0	1	0	0	0
1	349	26	23	0	0	0	23	8	2
5	50	24	9	0	1	5	4	0	0
4	22	17	12	0	0	0	0	0	0
2	11	26	13	0	0	0	0	0	0
26	73	108	22	37	4	2	4	10	2
13	8	131	14	4	0	0	1	0	0
2	4	0	0	0	0	2	0	0	0

ANI (%): 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100

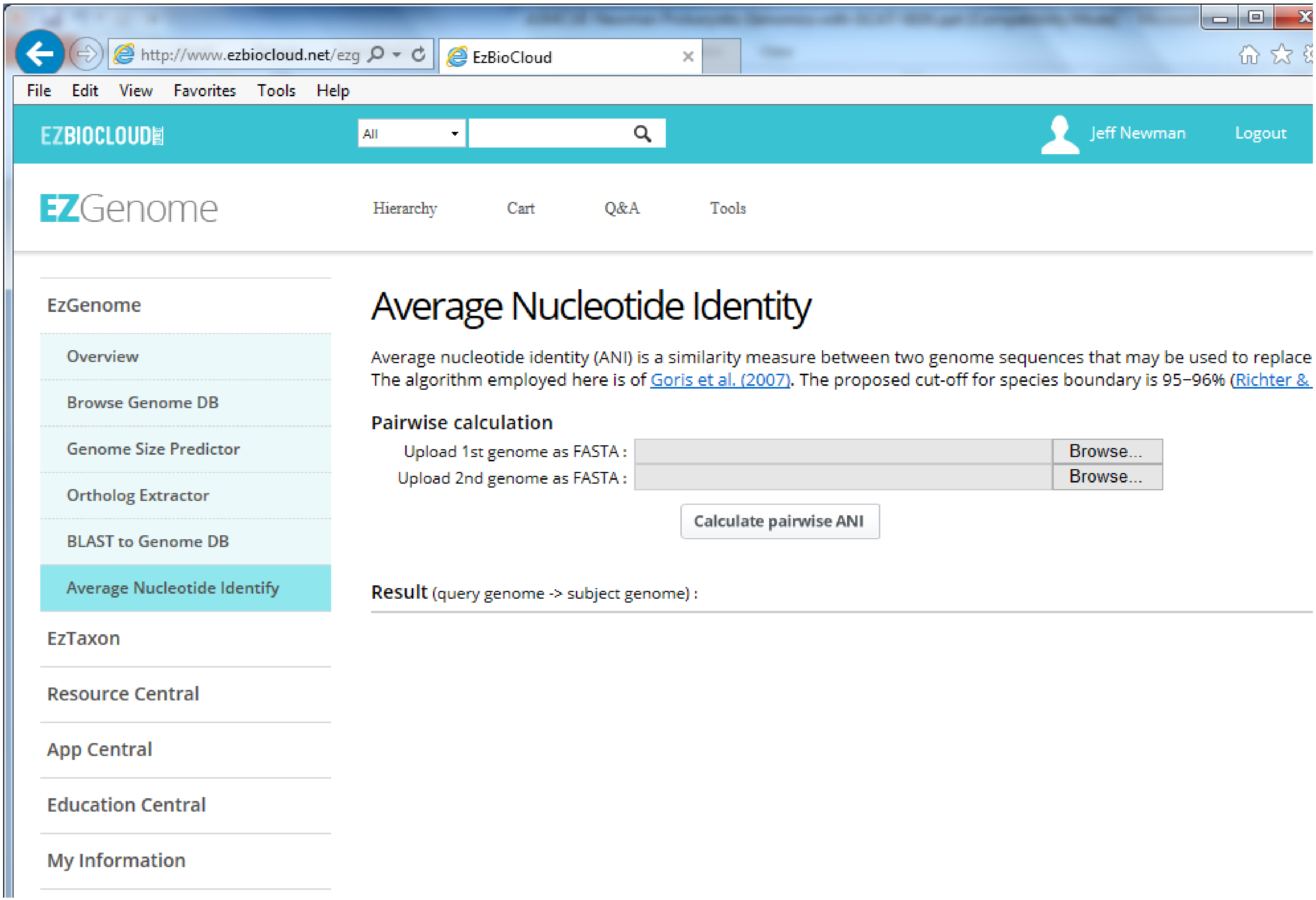
Count: 300 >, 200, 100, 0

Fig. 3. Association plot (a) and table (b) between ANI values and 16S rRNA gene sequence similarities. The number of strain pairs is displayed in each category square when divided by several intervals in 16S rRNA gene sequence similarity and 1.0 in ANI.

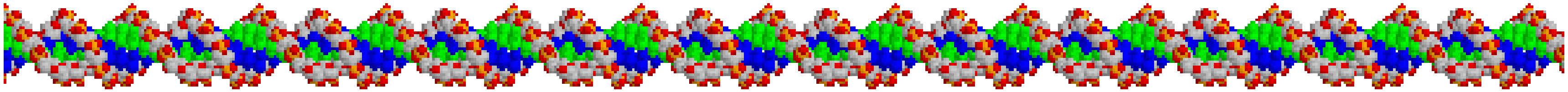
Determine Phylogenomic Metrics – Kostas Lab ANI Calculator



Determine Phylogenomic Metrics – Chun Lab EzGenome ANI Calculator



Genome-Genome Distance Calculator (GGDC) at the DSMZ calculates digital DDH



GGDC

Genome-to-Genome Distance Calculator

About GGDC 1.0 GGDC 2.0 FAQ Contact Legal Notice

About this service

The pragmatic species concept for Bacteria and Archaea is ultimately based on DNA-DNA hybridization (DDH). While enabling the taxonomist, in principle, to obtain an estimate of the overall similarity between the genomes of two strains, this technique is tedious and not easily be made reproducible between different labs. Furthermore, it cannot be used to incrementally built up a comparative database. Recent technological progress in the area of genome sequencing calls for bioinformatics methods to replace the wet-lab DDH by in-silico genome-to-genome comparison. This web service offers state-of-the-art methods for inferring whole-genome distances which are well able to mimic DDH. These distance functions can also cope with heavily reduced genomes and repetitive sequence regions. Some of them are also very robust against missing fractions of genomic information (due to incomplete genome sequencing). Our digitally derived genome-to-genome distances show a better correlation with 16S rRNA gene sequence distances than DDH values. Thus, this web service can be used for **genome-based species delineation**. Once you have obtained complete or incomplete, assembled genomes sequences, the use is easy: upload your sequence files in our distance calculation form and let our server calculate intergenomic distances for you. These are converted into similarity values analogous to DDH and sent to you via e-mail to support your **decision about the relatedness of your novel strain to known type strains**.

The GGDC has been developed entirely independently of the ANI ("average nucleotide identity") concept and is in no way based on it. Indeed, the core of GGDC, the GBDP program for calculating intergenomic distances, has been published *before* the first paper on ANI. GBDP conducts a couple of corrections that are not found in ANI, and in contrast to ANI GBDP does not split the sequences into sections of an arbitrary length of 1000 bp. In the studies listed below, GGDC yielded higher correlations with wet-lab DDH than ANI, and as of version 2.0 GGDC uses statistical models that considerably improve on the linear models used by ANI and earlier versions of GGDC. A practical advantage of GGDC over ANI is that GGDC operates on the same scale than wet-lab DDH values, which makes comparisons much easier. See the FAQ for details.

ANI & GGDC

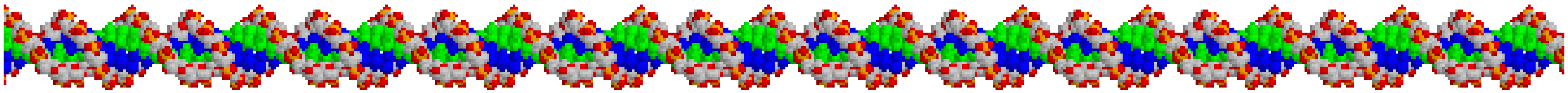
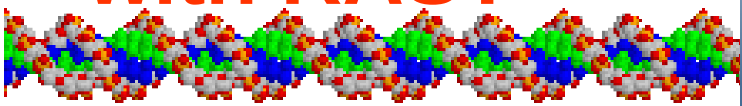


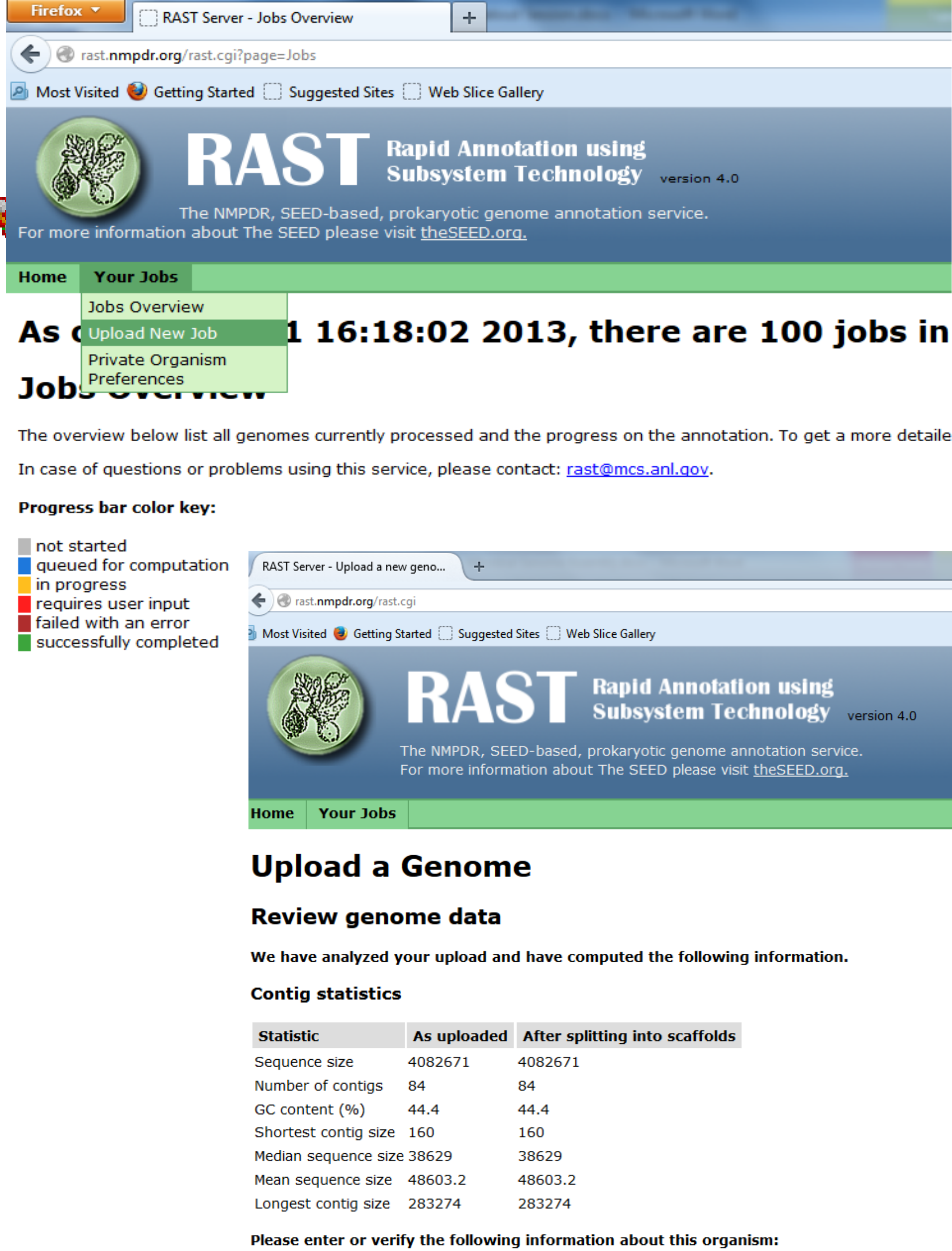
Table 2. Estimated DNA-DNA Hybridization (species threshold = 70%) calculated by GGDC (Meier-Kolthoff. et al, 2013) and Average Nucleotide Identity (ANI) (species threshold = 95%) calculated by Kostas lab ANI Calculator http://enve-omics.ce.gatech.edu/ani/

	Flavobacterium JRM	***Flavobacterium KMS***	***Flavobacterium hydatis***	***Flavobacterium hibernum***
Flavobacterium sp. JRM	**-**	**74.2**	**41.7**	**23.4**
Flavobacterium sp. KMS	**96.95**	**-**	**40.8**	**23.3**
Flavobacterium hydatis	**90.54**	**90.22**	**-**	**23.3**
Flavobacterium hibernum	**78.36**	**78.25**	**78.38**	**-**

Annotation with RAST



- http://rast.nmpdr.org/
- Upload genome as a .fasta file,
- Get GC mol% composition
- 1-24 hrs later…



RAST Annotation = Genes identified and classified by function

Genome	Pedobacter sp BMA
Domain	Sphingobactericeae
Taxonomy	Sphingobactericeae ; Pedobacter sp BMA
Neighbors	View closest neighbors
Size	5,046,646 bp
Number of Contigs (with PEGs)	36
Number of Subsystems	352
Number of Coding Sequences	4425
Number of RNAs	55

For each genome we offer a wide set of information to browse, compare and download.

Browse | Compare | Download | Annotate

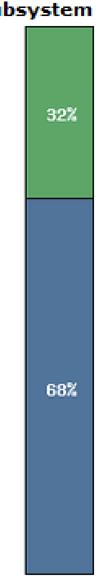
Compare the metabolic reconstruction of this organism to that of another organism.

Available comparisons are function based, sequence based or via KEGG. You can also BLAST against this organism.

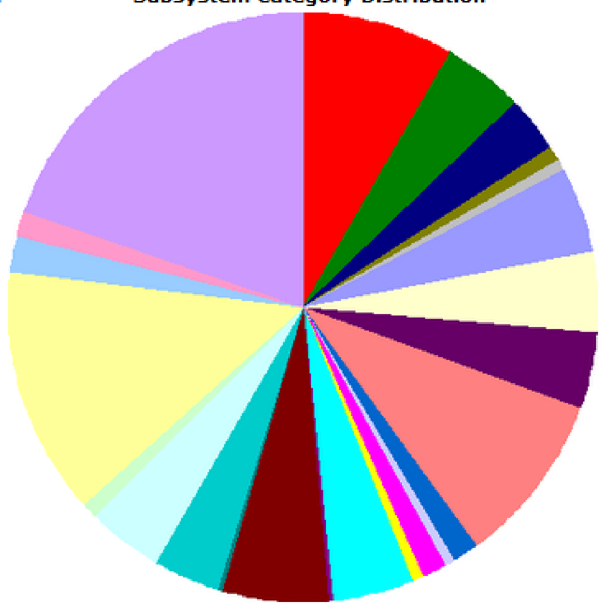
Subsystem Information

Subsystem Statistics | Features in Subsystems

Subsystem Coverage



Subsystem Category Distribution



Subsystem Feature Counts

- Cofactors, Vitamins, Prosthetic Groups, Pigments (170)
- Cell Wall and Capsule (90)
- Virulence, Disease and Defense (61)
- Potassium metabolism (14)
- Photosynthesis (0)
- Miscellaneous (15)
- Phages, Prophages, Transposable elements, Plasmids (1)
- Membrane Transport (90)
- Iron acquisition and metabolism (5)
- RNA Metabolism (93)
- Nucleosides and Nucleotides (85)
- Protein Metabolism (185)
- Cell Division and Cell Cycle (30)
- Motility and Chemotaxis (11)
- Regulation and Cell signaling (29)
- Secondary Metabolism (7)
- DNA Metabolism (94)
- Regulons (6)
- Fatty Acids, Lipids, and Isoprenoids (112)
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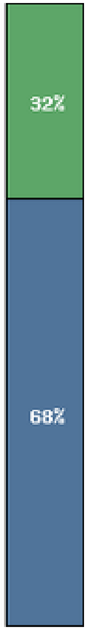
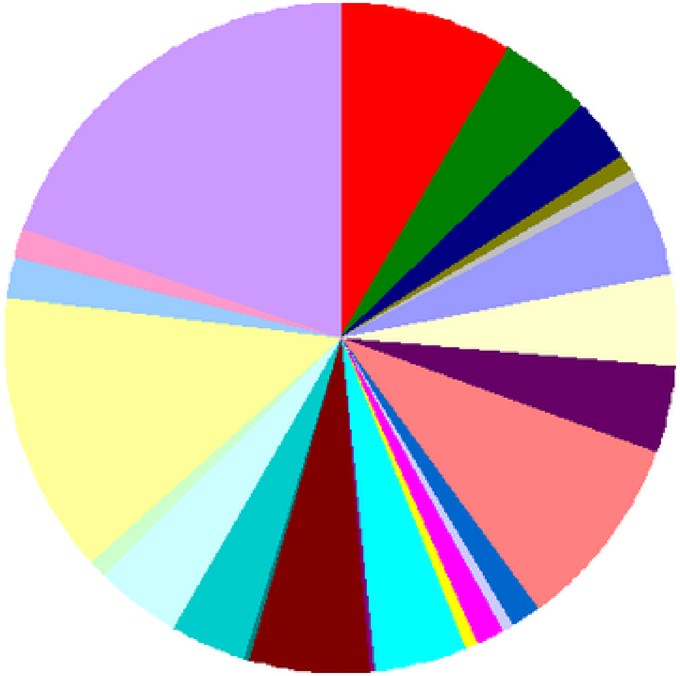
- Respiration (72)
- Stress Response (86)
- Metabolism of Aromatic Compounds (13)
- Amino Acids and Derivatives (276)
- Sulfur Metabolism (36)
- Phosphorus Metabolism (31)
- Carbohydrates (382)

Subsystem Coverage



Subsystem Category Distribution



Many genes assigned to expandable subsystems

Subsystem Feature Counts

- Cofactors, Vitamins, Prosthetic Groups, Pigments (170)
- Cell Wall and Capsule (90)
- Virulence, Disease and Defense (61)
  - Adhesion (0)
  - Toxins and superantigens (0)
  - Bacteriocins, ribosomally synthesized antibacterial peptides (0)
  - Resistance to antibiotics and toxic compounds (46)
    - Copper homeostasis (3)
    - Cobalt-zinc-cadmium resistance (17)
    - Multidrug Resistance, Tripartite Systems Found in Gram Negative Bacteria (6)
    - Zinc resistance (2)
    - Mercuric reductase (1)
    - Arsenic resistance (4)
    - Resistance to fluoroquinolones (4)
    - Beta-lactamase (8)
    - Resistance to chromium compounds (1)
  - Virulence, Disease and Defense - no subcategory (0)
  - Detection (0)
  - Invasion and intracellular resistance (15)
- Potassium metabolism (14)
- Photosynthesis (0)
- Miscellaneous (15)
- Phages, Prophages, Transposable elements, Plasmids (1)
  - Phage family-specific subsystems (0)
  - Transposable elements (0)
  - Phages, Prophages (1)
  - Phages, Prophages, Transposable elements, Plasmids - no subcategory (0)
  - Pathogenicity islands (0)
  - Gene Transfer Agent (GTA) (0)
  - Plasmid related functions (0)
- Membrane Transport (90)
- Iron acquisition and metabolism (5)
- RNA Metabolism (93)
- Nucleosides and Nucleotides (85)
- Protein Metabolism (185)
- Cell Division and Cell Cycle (30)
- Motility and Chemotaxis (11)
- Regulation and Cell signaling (29)
- Secondary Metabolism (7)
- DNA Metabolism (94)
- Regulons (6)
- Fatty Acids, Lipids, and Isoprenoids (112)
  - Phospholipids (20)
  - Triacylglycerols (0)
  - Fatty acids (36)
  - Fatty Acids, Lipids, and Isoprenoids - no subcategory (11)
  - Isoprenoids (45)
    - Myxoxanthophyll biosynthesis in Cyanobacteria (1)
    - Carotenoids (11)
    - Isoprenoinds for Quinones (6)
    - Isoprenoid Biosynthesis (14)
    - Polyprenyl Diphosphate Biosynthesis (4)
    - Nonmevalonate Branch of Isoprenoid Biosynthesis (7)

Phenotype Comparisons

B

	Chryseo. populense	Chryseo. hispalense	Chryseo. wanjuense	Chryseo. daecheongense
neg control	19	34	25	38
dextrin	100	100	100	100
D-maltose	82	96	97	93
D-trehalose	95	19	99	98
D-cellobiose	8	21	27	35
gentiobiose	99	100	100	99
sucrose	11	19	22	99
D-turanose	8	14	8	16
stachyose	9	18	15	28
pos control	97	98	97	98
pH 6	96	98	96	97
pH 5	93	96	92	96
D-raffinose	14	16	19	26
α-D-lactose	10	11	20	18
D-melibiose	11	22	26	38
β-methyl-D-glucoside	8	14	12	18
D-salicin	7	8	6	11
N-acetyl-D-glucosamine	7	10	17	18
N-acetyl-β-D-mannosamine	7	10	15	21
N-acetyl-D-galactosamine	9	10	10	20
N-acetyl neuraminic acid	11	23	24	37
1% NaCl	94	95	93	94
4% NaCl	13	69	8	11
8% NaCl	17	19	10	15
α-D-glucose	82	93	99	91
D-mannose	98	84	95	88
D-fructose	99	98	99	21
D-galactose	13	12	14	23
3-methyl glucose	6	8	10	9
D-fucose	12	10	17	28
L-fucose	11	16	12	24
L-rhamnose	29	89	59	15

	Chryseo. populense	Chryseo. hispalense	Chryseo. wanjuense	Chryseo. daecheongense
inosine	9	11	8	9
1% Na-lactate	94	94	93	92
fusidic acid	11	10	9	7
D-serine	16	12	11	13
D-sorbitol	14	18	18	29
D-mannitol	9	10	15	14
D-arabitol	9	9	11	14
myo-inositol	11	11	22	26
glycerol	77	8	93	81
D-glucose-6-PO4	11	19	16	13
D-fructose-6-PO4	18	22	17	22
D-aspartic acid	7	8	4	4
D-serine	9	8	4	3
troleandomycin	9	9	38	11
rifamycin SV	95	90	95	95
minocycline	16	12	16	12
gelatin	100	100	100	100
glycyl-L-proline	91	42	81	88
L-alanine	8	8	4	3
L-arginine	12	22	18	59
L-aspartic acid	27	98	57	95
L-glutamic acid	97	99	90	99
L-histidine	12	13	9	14
L-pyroglutamic acid	10	11	12	8
L-serine	51	8	4	9
lincomycin	95	9	94	95
guanidine HCl	21	8	86	6
niaproof 4	12	11	9	8
pectin	22	97	44	90
D-galacturonic acid	95	97	78	18
L-galacturonic acid lactone	8	8	5	4
D-gluconic acid	9	11	10	9

	Chryseo. populense	Chryseo. hispalense	Chryseo. wanjuense	Chryseo. daecheongense
D-glucuronic acid	19	14	23	23
glucuronamide	17	16	10	13
mucic acid	10	10	10	7
quinic acid	11	11	13	7
D-saccharic acid	12	11	12	8
vancomycin	12	7	9	26
tetrazolium violet	98	43	91	85
tetrazolium blue	98	99	98	66
p-hydroxy-phenylacetic acid	13	12	8	10
methyl pyruvate	11	8	6	3
D-lactic acid methyl ester	11	8	8	5
L-lactic acid	12	8	16	7
citric acid	11	13	12	17
α-keto-glutaric acid	11	8	57	6
D-malic acid	10	8	11	6
L-malic acid	12	11	14	6
bromo-succinic acid	9	8	4	3
nalidixic acid	14	9	13	9
LiCl	14	73	11	6
K-tellurite	95	52	96	97
tween-40	94	95	95	98
γ-amino-butyric acid	14	8	16	9
α-hydroxy-butyric acid	17	10	13	6
β-hydroxy-D,L-butyric acid	15	9	20	7
α-keto-butyric acid	16	8	8	3
acetoacetic acid	75	86	72	82
propionic acid	14	8	8	8
acetic acid	97	94	99	99
formic acid	16	8	12	61
aztreonam	97	98	96	97
Na-butyrate	36	85	15	32
Na bromate	20	12	21	21

C

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 (0)

One-carbon Metabolism (40)
- Serine-glyoxylate cycle (37)
- One-carbon metabolism by tetrahydropterines (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)
- Acetoin, butanediol 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)
- Cellulosome (13)

Monosaccharides (66)
- Mannose Metabolism (11)
- D-ribose utilization (3)
- Xylose utilization (12)
- Deoxyribose and Deoxynucleoside Catabolism (7)
- 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)

Glycoside hydrolases (0)

One-carbon Metabolism (38)
- Serine-glyoxylate cycle (34)
- One-carbon metabolism by tetrahydropterines (4)

Organic acids (1)
- Lactate utilization (1)

Fermentation (31)
- Butanol Biosynthesis (16)
- Acetyl-CoA fermentation to Butyrate (15)

CO2 fixation (1)
- CO2 uptake, carboxysome (1)

Sugar alcohols (0)

Carbohydrates - no subcategory (0)

Polysaccharides (19)
- Glycogen metabolism (5)
- Cellulosome (14)

Monosaccharides (62)
- Mannose Metabolism (8)
- D-ribose utilization (3)
- Xylose utilization (10)
- Deoxyribose and Deoxynucleoside Catabolism (7)
- L-Arabinose utilization (4)
- D-Galacturonate and D-Glucuronate Utilization (30)

RAST

The SEED Viewer

SEEKquence

Seed Viewer Sequence Based Comparison Tool

Genome	Pedobacter sp BMA
Domain	Sphingobactericeae
Taxonomy	Sphingobactericeae ; Pedobacter sp BMA
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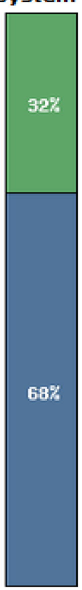
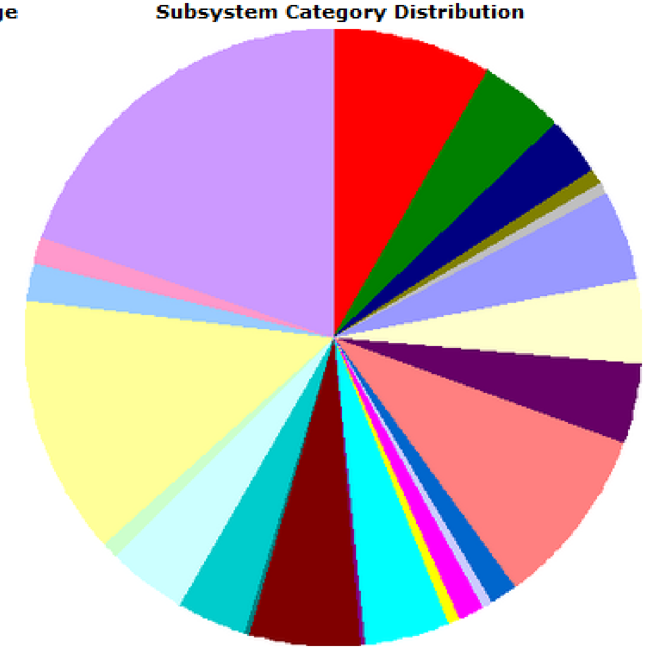


Subsystem Category Distribution



Subsystem Feature Counts

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- Virulence, Disease and Defense (61)
- Potassium metabolism (14)
- Photosynthesis (0)
- Miscellaneous (15)
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- Sulfur Metabolism (36)
- Phosphorus Metabolism (31)
- Carbohydrates (382)

RAST – Sequence based comparison tool to ID orthologs

http://rast.nmpdr.org/seedviewer.cgi | About this service | Taylor Anspach - Outlook Web... | RAST Server - Job Details | Seed Viewer - Multi-Genom... | Newman Lab Orthology Calcul...

change organism selection

You chose to compute data for the following organisms:

Reference	Sphingomonas melonis C3 (1090316.6)
Comparison Organism 1	Novosphingobium aromaticivorans DSM 12444 (279238.21) BlastDotPlot
Comparison Organism 2	Novosphingobium nitrogenifigens DSM 19370 (983920.4) BlastDotPlot
Comparison Organism 3	Novosphingobium nitrogenifigens Y88, DSM 19370 (983920.5) BlastDotPlot
Comparison Organism 4	Sphingomonas melonis C3 (1090316.6) BlastDotPlot
Comparison Organism 5	Sphingomonas melonis DAPP-PG 224 (1090320.4) BlastDotPlot
Comparison Organism 6	Sphingomonas melonis FR1 (1090317.3) BlastDotPlot
Comparison Organism 7	Novosphingobium aromaticivorans (48935.1) BlastDotPlot
Comparison Organism 8	Sphingomonas wittichii RW1 (392499.4) BlastDotPlot
Comparison Organism 9	Sphingopyxis alaskensis RB2256 (317655.9) BlastDotPlot

Percent protein sequence identity

Bidirectional best hit	100	99.9	99.8	99.5	99	98	95	90	80	70	60	50	40	30	20	10
Unidirectional best hit	100	99.9	99.8	99.5	99	98	95	90	80	70	60	50	40	30	20	10

export table | clear all filters

display 30 items per page

«first «prev | displaying 2 - 31 of 3706 | next» last»

percent identity 279238.21 <
percent identity 983920.5 <
percent identity 1090320.4 <
percent identity 48935.1 <
percent identity 317655.9 <
percent identity 983920.4 <
percent identity 1090316.6 <
percent identity 1090317.3 <
percent identity 392499.4 <

1090316.6			279238.21			983920.4			983920.5			1090316.6		
Contig	Gene	Length	Hit	Contig	Gene	Hit	Contig	Gene	Hit	Contig	Gene	Hit	Contig	Gene
1	2	97	-			-			-			bi	1	16
1	3	184	uni	2	3527	-			-			bi	1	15
1	4	155	-			-			-			bi	1	14
1	5	510	-			-			-			-		
1	6	234	uni	1	1499	uni	9	1438	uni	61	3817	uni	2	3556
1	7	504	uni	1	1498	uni	1	1	uni	61	3816	uni	2	3555
1	8	1040	-			bi	1	292	bi	30	1865	-		
1	9	178	-			-			-			-		
1	10	292	-			bi	1	290	bi	30	1867	-		
1	11	591	-			bi	1	289	bi	30	1868	-		
1	12	612	-			bi	1	288	bi	30	1869	bi	1	764
1	13	55	-			-			-			-		
1	14	155	-			-			-			bi	1	4
1	15	184	uni	2	3527	-			-			bi	1	3
1	16	97	-			-			-			bi	1	2
1	17	90	-			-			-			bi	1	1
1	18	493	-			bi	4	1156	bi	8	207	bi	1	1320
1	19	310	bi	1	3176	bi	5	1244	bi	29	1618	uni	1	749
1	20	266	bi	1	3284	bi	43	3562	bi	44	3313	-		
1	21	478	bi	1	3274	bi	43	3551	bi	44	3302	uni	1	973
1	22	250	-			-			-			-		
1	23	1514	bi	1	3275	bi	43	3552	bi	44	3303	-		
1	24	64	-			-			-			-		
1	25	225	bi	1	3175	bi	5	1243	bi	29	1617	uni	1	1675
1	26	121	-			bi	2	742	bi	34	2391	-		
1	27	788	bi	1	712	bi	13	1972	bi	20	1089	-		
1	28	372	bi	1	711	bi	13	1973	bi	20	1088	-		
1	29	113	bi	1	837	uni	12	1606	uni	23	1446	uni	1	1516
1	30	122	bi	1	709	bi	13	1974	bi	20	1087	-		
1	31	68	bi	1	708	bi	13	1975	bi	20	1086	-		

1090316.6	1090320.4			1090317.3			48935.1			392499.4			317655.9		
Gene	Hit	Contig	Gene	Hit	Contig	Gene	Hit	Contig	Gene	Hit	Contig	Gene	Hit	Contig	Gene
2	-			uni	1	1542	-			-			-		
3	uni	3	3836	uni	1	1543	uni	1	26	uni	2	332	-		
4	-			uni	1	1544	-			-			-		
5	-			bi	1	1545	-			-			-		
6	-			uni	1	1546	uni	114	1298	uni	1	14	uni	1	394
7	-			uni	1	1547	uni	114	1299	uni	1	13	uni	1	395
8	-			bi	1	1549	-			-			-		
9	-			bi	1	1550	-			-			-		
10	-			bi	1	1551	-			-			-		
11	-			bi	1	1552	-			-			-		
12	-			bi	1	1553	-			-			-		
13	-			bi	1	1554	-			-			-		
14	-			bi	1	1544	-			-			-		
15	uni	3	3836	bi	1	1543	uni	1	26	bi	2	332	-		
16	-			bi	1	1542	-			-			-		
17	-			uni	1	1541	-			-			-		
18	uni	1	3373	bi	1	1559	-			bi	3	2664	uni	1	48
19	bi	1	666	bi	1	1560	bi	49	431	bi	3	987	bi	1	2062
20	bi	1	665	bi	1	1561	bi	167	3004	bi	3	986	bi	1	2063
21	bi	1	664	bi	1	1562	bi	167	3014	bi	3	985	bi	1	2064
22	bi	1	663	bi	1	1563	-			-			bi	1	2065
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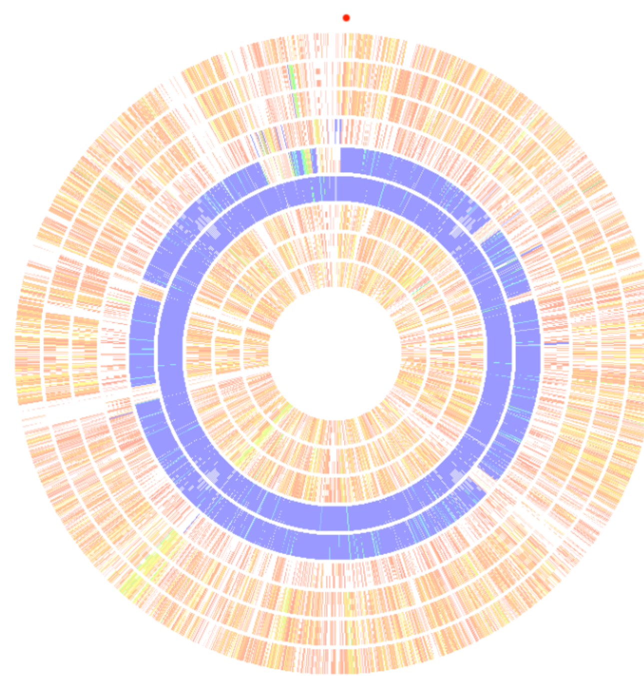
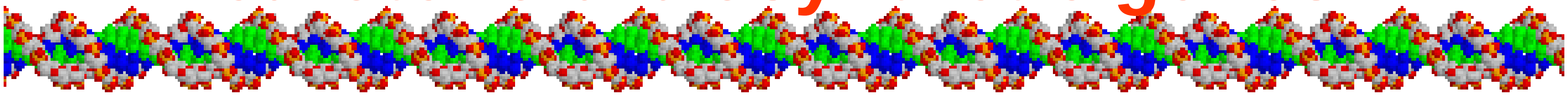
24	bi	1	661	bi	1	1565	-			-			-		
25	bi	1	660	bi	1	1566	bi	49	430	bi	3	4732	bi	1	2068
26	bi	1	659	bi	1	1567	-			-			bi	1	1159
27	bi	1	658	bi	1	1568	bi	140	1928	bi	3	2636	bi	1	2648
28	bi	1	657	bi	1	1569	bi	140	1927	bi	3	2637	bi	1	2647
29	bi	1	656	bi	1	1570	bi	174	3470	bi	3	2638	bi	1	684
30	bi	1	655	bi	1	1571	bi	140	1925	bi	3	2639	bi	1	2796
31	bi	1	654	bi	1	1572	bi	140	1924	bi	3	2640	bi	1	2797



10:03 PM
11/10/2013

C.populense lacks carotenoid biosynthetic genes



C.hispalense *C.populense*

491205.4			558151.4			1121286.3			236814.3			525257.7			1121287.3			1218103.4			558152.3			445961.3			59732.8			307480.3		
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all				all			all			all			all			all			all			all			all			all			all	
8	991	794	-			bi	4	1671	bi	5	858	bi	38	3567	bi	4	1495	-			-			-			bi	108	4042	-		
8	992	637	-			bi	4	1670	bi	5	859	bi	38	3568	bi	4	1496	-			-			bi	2	270	bi	108	4041	-		
8	993	482	-			bi	4	1669	bi	5	860	bi	38	3569	bi	4	1497	-			-			bi	2	271	bi	108	4040	-		
8	994	362	-			bi	4	1668	bi	5	861	bi	38	3570	bi	4	1498	-			-			bi	2	272	bi	108	4039	-		
8	995	324	-			bi	4	1667	bi	5	862	bi	38	3571	bi	4	1499	-			-			bi	2	273	bi	108	4038	-		
8	996	678	-			bi	4	1666	bi	5	863	bi	38	3572	bi	4	1501	-			-			bi	2	274	bi	108	4036	-		
8	997	199	-			-			bi	5	1990	bi	38	3573	bi	4	1502	-			-			-			-			-		
8	998	291	bi	1	523	bi	5	2037	bi	5	1991	bi	38	3574	bi	4	1503	bi	19	4242	bi	37	2234	bi	11	2704	bi	34	1232	bi	27	4951
8	999	396	bi	1	50	bi	4	1664	bi	5	867	bi	38	3575	bi	4	1504	-			bi	82	3758	-			bi	108	4032	-		
8	1000	351	bi	1	51	bi	4	1663	bi	5	868	bi	38	3576	bi	4	1505	-			bi	82	3759	-			bi	108	4031	-		
8	1001	150	-			bi	17	3765	bi	5	114	bi	7	907	bi	4	1512	bi	7	1747	-			bi	13	303	-					
8	1002	223	-			bi	17	3766	bi	5	2174	bi	7	970	bi	4	1513	bi	7	1762	-			bi	17	341	-			i	19	4081
8	1003	490	uni	11	3029	bi	17	3767	bi	5	2173	bi	7	953	bi	4	1514	bi	7	1563	uni	24	1021	bi	17	341	-			i	19	4080
8	1004	279	-			bi	17	3768	bi	5	2172	bi	7	954	bi	4	1515	bi	7	1564	-			bi	17	341	-			i	19	4079
8	1005	152	-			bi	17	3769	bi	5	2170	bi	7	956	bi	4	1516	bi	7	1566	-			bi	17	340	-			i	19	4077
8	1006	238	-			bi	17	3770	bi	5	2169	bi	7	957	bi	4	1517	bi	7	1567	-			bi	17	340	-			i	19	4076
8	10[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		-			-			-			-			-								
8	10[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2234	bi	1	511	bi	4	1403	bi	2	273	bi	75	3573	bi	5	753	bi	16	370	bi	2	1192
8	10[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2241	bi	10	1314	bi	4	1396	bi	10	2705	uni	36	2185	bi	17	3369	uni	102	3947	bi	5	1891
8	1010	160	-			bi	4	1579	-			bi	42	3938	bi	4	1519	bi	10	2602	bi	28	1303	-			-			bi	5	1956
8	1011	78	-			bi	4	1578	bi	5	97	bi	4	651	bi	4	1520	-			-			bi	13	2986	-			bi	5	1967
8	1012	966	uni	3	1058	-			bi	5	877	-			bi	4	1521	uni	11	2751	uni	21	825	bi	17	3444	-			bi	5	1982
8	1013	204	uni	1	225	bi	4	1577	uni	5	2523	bi	27	2599	bi	4	1522	bi	10	2634	bi	28	1304	bi	17	3431	uni	20	644	bi	5	1970
8	1014	73	-			bi	17	3764	bi	5	886	-			bi	4	1523	-			-			bi	13	3025	bi	25	886	-		
8	1015	52	-			-			-			-			-			-			-			-			-			-		
8	1016	77	-			bi	17	3771	bi	5	1964	bi	21	2217	bi	4	1525	bi	19	4260	-			-			bi	72	2999	bi	5	1968
8	1017	332	uni	12	4297	bi	17	3772	bi	5	2229	bi	42	3977	bi	4	1526	bi	10	2555	bi	7	264	bi	2	249	bi	2	21	uni	11	3903

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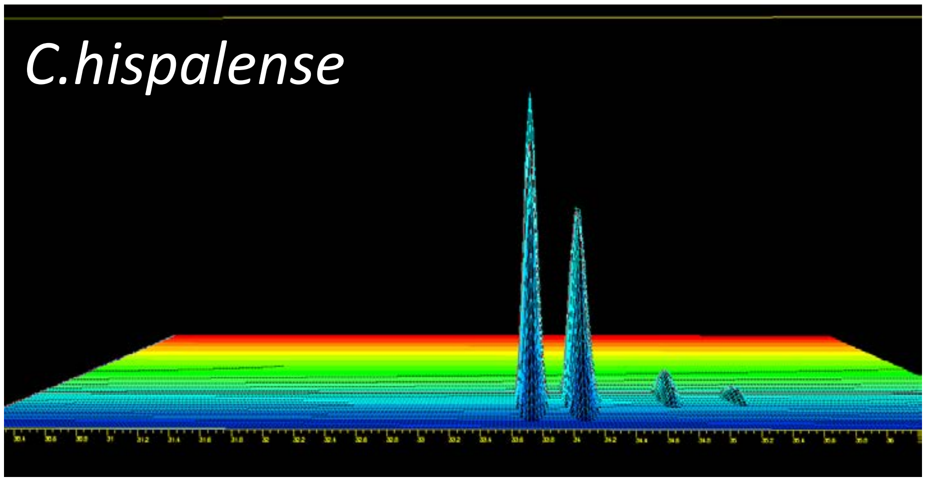
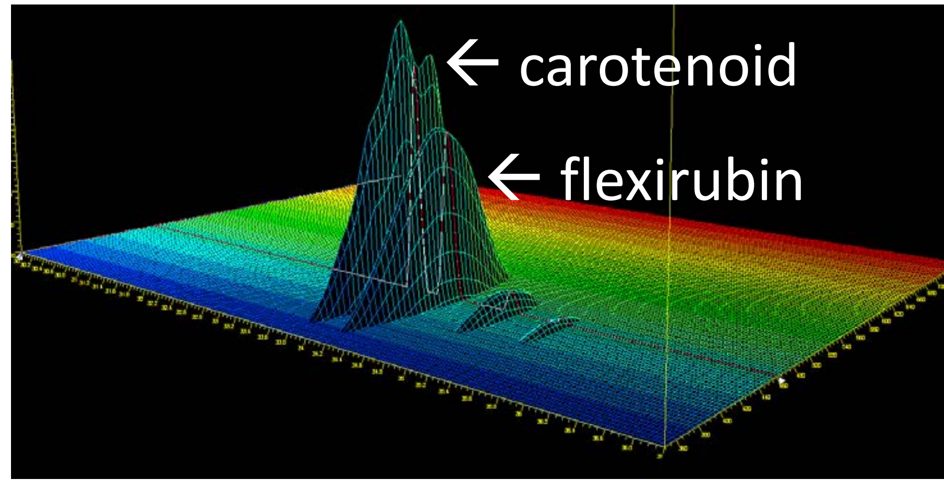
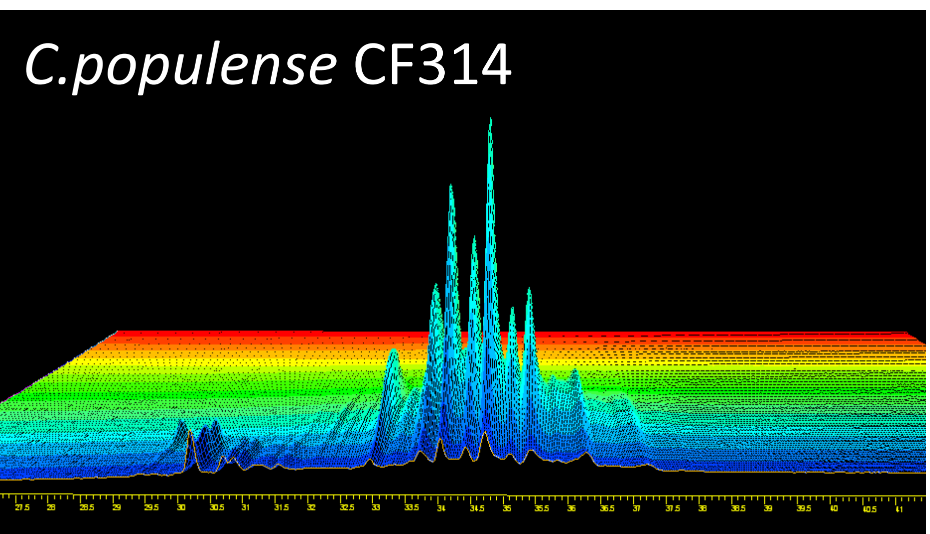
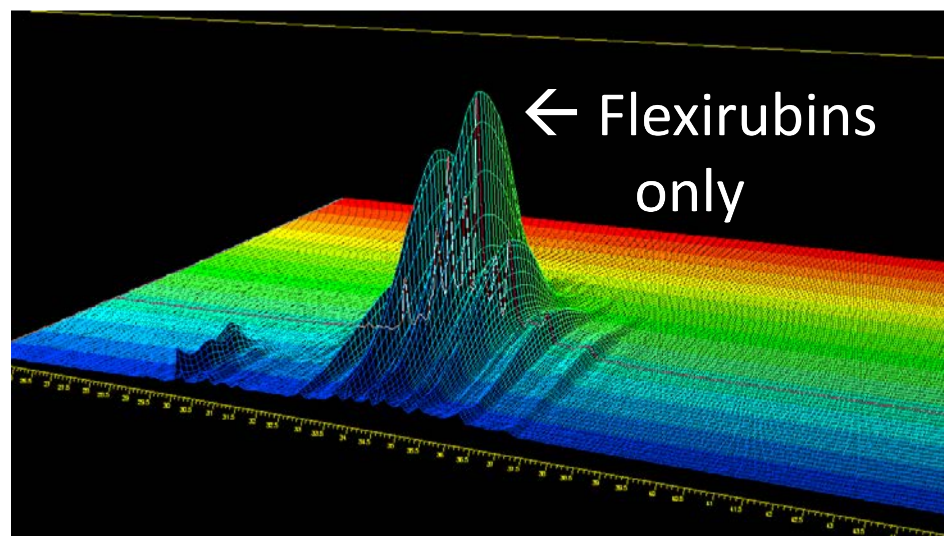
Explain phenotypic differences – e.g. Pigment "Landscapes"









Sequence-Based Comparison color codes similarity

You chose to compute data for the following organisms:

Reference	Chryseobacterium hispalense DSM 25574 (491205.4)
Comparison Organism 1	Chryseobacterium gleum F93, ATCC 35910 (525257.7) BlastDotPlot
Comparison Organism 2	Chryseobacterium sp. CF314 (1144316.4) BlastDotPlot

Percent protein sequence identity

Bidirectional best hit	100	99.9	99.8	99.5	99	98	95	90	80	70	60	50	40	30	20	10
Unidirectional best hit	100	99.9	99.8	99.5	99	98	95	90	80	70	60	50	40	30	20	10

export table   clear all filters

display 30 items per page

«first «prev   displaying 3028 - 3057 of 4006   next» last»

percent identity 525257.7 <   percent identity 1144316.4 <

491205.4			525257.7			1144316.4		
Contig (all)	Gene	Length	Hit (all)	Contig (all)	Gene	Hit (all)	Contig (all)	Gene
21	3028	360	bi	1	241	bi	22	1525
21	3029	270	bi	1	240	uni	51	2777
21	3030	680	bi	1	239	uni	2	181
21	3031	92	bi	1	238	uni	14	1009
21	3032	580	bi	1	237	uni	49	2655
21	3033	298	bi	1	236	bi	2	183
21	3034	293	bi	1	235	bi	2	184
21	3035	249	bi	1	234	uni	1	1
21	3036	440	bi	1	233	bi	2	185
21	3037	1455	bi	1	232	bi	2	186
21	3038	41	bi	1	231	-		
21	3039	208	bi	1	230	bi	69	3277
21	3040	348	bi	1	229	uni	3	200
21	3041	92	bi	1	228	uni	59	2913
21	3042	258	bi	1	227	-		
21	3043	158	bi	1	[illegible]	[illegible]	[illegible]	[illegible]
21	3044	110	bi	1	[illegible]	[illegible]	[illegible]	[illegible]
21	3045	408	bi	1	[illegible]	[illegible]	[illegible]	[illegible]
21	3046	106	bi	1	[illegible]	[illegible]	[illegible]	[illegible]
21	3047	392	bi	1	[illegible]	[illegible]	[illegible]	[illegible]

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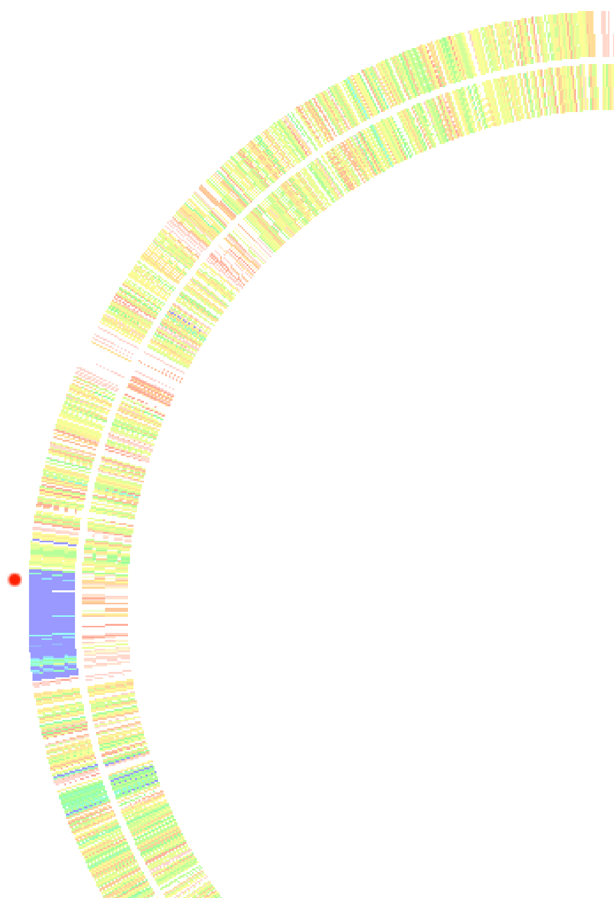
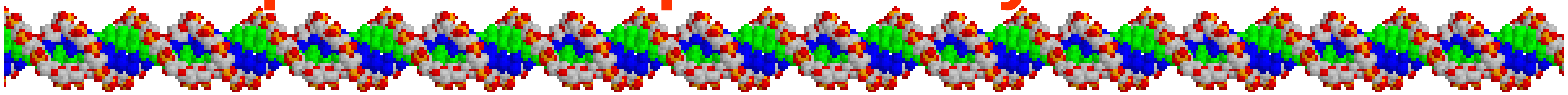


Sequence Based Comparison provides protein seq similarity → AAI

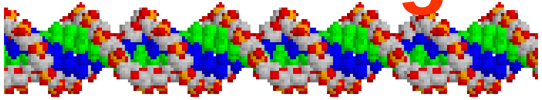


Microsoft Excel - Fsucc Venn for poster.xlsx

Contig	Length	Gene id	Gene	function	Hit	Contig	Gene	Gene id	percent	function	Hit	Contig	Gene	Gene id	percent	function
22	149	fig|1450525.4.pe	3118	hypothetical protein	-				0		bi	1	2298	fig|37668	61.9	hypothetical protein
23	162	fig|1450525.4.pe	3124	Pectate lyase (EC 4.2.2.2)	-				0		bi	1	2696	fig|37668	73.91	Pectate lyase (EC 4.2.2.2)
25	72	fig|1450525.4.pe	3173	Glucosamine-6-phosphate deaminase (EC 3.5.99.6)	-				0		bi	1	4817	fig|37668	91.55	Glucosamine-6-phosphate deaminase (EC 3.5.99
26	408	fig|1450525.4.pe	3177	Gluconate permease, Bsu4004 homolog	-				0		bi	1	700	fig|37668	75.68	Gluconate permease, Bsu4004 homolog
26	156	fig|1450525.4.pe	3178	Endoribonuclease L-PSP	-				0		bi	1	701	fig|37668	92.26	Endoribonuclease L-PSP
26	216	fig|1450525.4.pe	3180	4-Hydroxy-2-oxoglutarate aldolase (EC 4.1.3.16) @	-				0		bi	1	703	fig|37668	73.36	4-hydroxy-2-oxoglutarate aldolase (EC 4.1.3.16)
26	372	fig|1450525.4.pe	3181	low-specificity D-threonine aldolase	-				0		bi	1	704	fig|37668	64.42	low-specificity D-threonine aldolase
26	346	fig|1450525.4.pe	3182	Membrane dipeptidase (EC 3.4.13.19)	-				0		bi	1	705	fig|37[illegible]8	87.54	Membrane dipeptidase (EC 3.4.13.19)
26	259	fig|1450525.4.pe	3183	Transcriptional repressor of the fructose operon, D	-				0		bi	1	706	[illegible]	86.77	Transcriptional repressor of the fructose operon
27	245	fig|1450525.4.pe	3188	Transcriptional regulator, AraC family	-				0		bi	1	4563	fig|3[illegible]68	37.68	regulatory protein; PcrR
28	68	fig|1450525.4.pe	3197	hypothetical protein	-				0		bi	1	1909	fig|37668	36.67	hypothetical protein
29	127	fig|1450525.4.pe	3205	hypothetical protein	-				0		bi	1	2828	fig|37668	40.54	hypothetical protein
32	228	fig|1450525.4.pe	3232	hypothetical protein	-				0		bi	1	769	fig|37668	34.87	hypothetical protein
38	38	fig|1450525.4.pe	3260	Ribonucleotide reductase of class Ia (aerobic), bet	-				0		bi	1	4302	fig|37668	89.19	Ribonucleotide reductase of class Ia (aerobic), b
39	94	fig|1450525.4.pe	3261	hypothetical protein	-				0		bi	1	4967	fig|37668	61.96	hypothetical protein
1	258	fig|1450525.4.pe	38	Possible restriction endonuclease	-				0		-				0	
1	274	fig|1450525.4.pe	56	Mobile element protein	-				0		-				0	
1	484	fig|1450525.4.pe	171	Predicted transcriptional regulator containing an I	-				0		-				0	
1	232	fig|1450525.4.pe	190	Sll8048 protein	-				0		-				0	
1	1568	fig|1450525.4.pe	191	Type I restriction-modification system, M subunit	-				0		-				0	
1	317	fig|1450525.4.pe	202	COG1242: Predicted Fe-S oxidoreductase	-				0		-				0	
1	257	fig|1450525.4.pe	246	probable integral membrane protein Cj1166c	-				0		-				0	
1	163	fig|1450525.4.pe	247	FIG001826: putative inner membrane protein	-				0		-				0	
1	829	fig|1450525.4.pe	262	Succinoglycan biosynthesis protein	-				0		-				0	
2	558	fig|1450525.4.pe	303	Formate--tetrahydrofolate ligase (EC 6.3.4.3)	-				0		-				0	
2	364	fig|1450525.4.pe	373	Cyanophycinase (EC 3.4.15.6)	-				0		-				0	
2	331	fig|1450525.4.pe	384	Homoserine kinase (EC 2.7.1.39)	-				0		-				0	
2	461	fig|1450525.4.pe	402	Type I restriction-modification system, specificity s	-				0		-				0	
2	76	fig|1450525.4.pe	432	Helix-turn-helix motif	-				0		-				0	
2	319	fig|1450525.4.pe	434	HipA protein	-				0		-				0	
2	68	fig|1450525.4.pe	448	DNA-binding domain of ModE	-				0		-				0	
2	172	fig|1450525.4.pe	451	Ubiquinol-cytochrome C reductase iron-sulfur sub	-				0		-				0	
2	350	fig|1450525.4.pe	454	Nitrate/nitrite transporter	-				0		-				0	
2	501	fig|1450525.4.pe	460	Cytochrome c552 precursor (EC 1.7.2.2)	-				0		-				0	
2	193	fig|1450525.4.pe	461	Cytochrome c nitrite reductase, small subunit NrfH	-				0		-				0	
2	79	fig|1450525.4.pe	475	B12 binding domain / kinase domain / Methylmalc	-				0		-				0	
2	119	fig|1450525.4.pe	490	Helix-turn-helix motif	-				0		-				0	

JOURNAL OF BACTERIOLOGY, Sept. 2005, p. 6258–6264 Vol. 187, No. 18
0021-9193/05/$08.00+0 doi:10.1128/JB.187.18.6258–6264.2005

AAI has broader range



Towards a Genome-Based Taxonomy for Prokaryotes

Konstantinos T. Konstantinidis[1,2] and James M. Tiedje[1,2,3]*

Center for Microbial Ecology[1] and Departments of Crop and Soil Sciences[2] and Microbiology and Molecular Genetics,[3] Michigan State University, East Lansing, Michigan

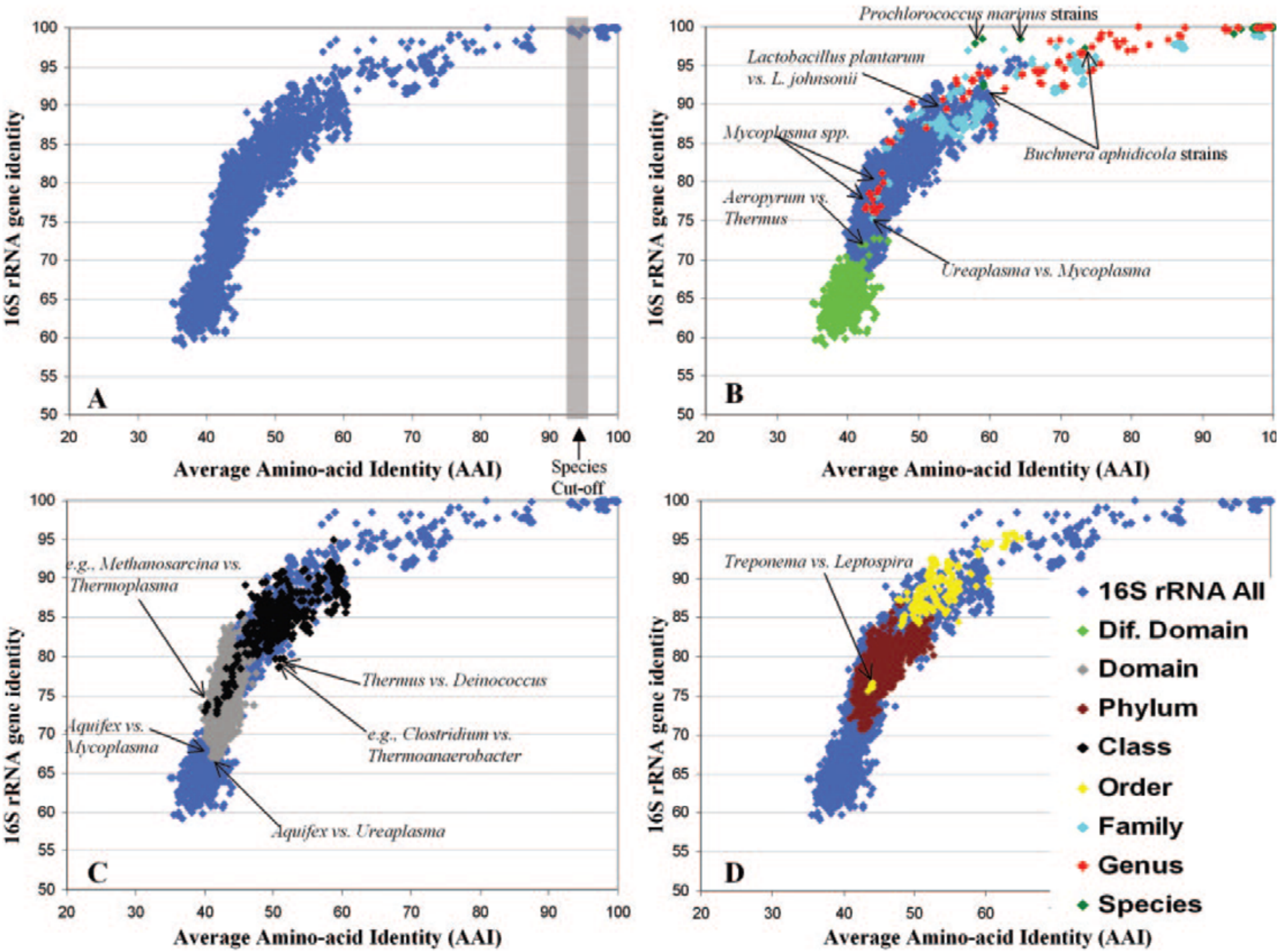
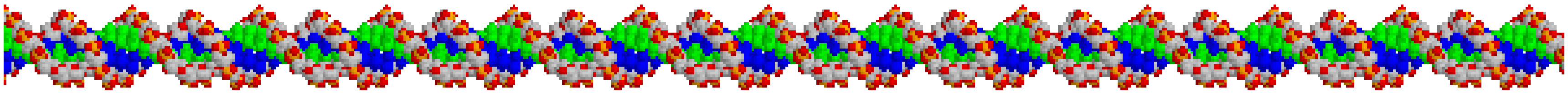


FIG. 3. Relationships between 16S rRNA, AAI, and taxonomic information for the 175 sequenced genomes. Each dot represents a compa

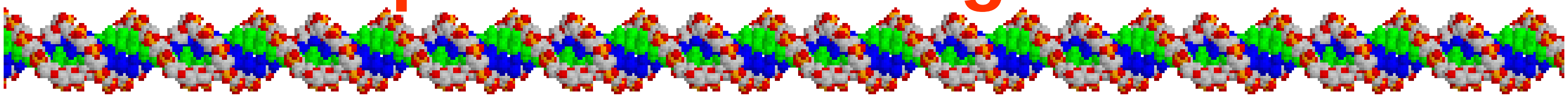
Different genera in same family have AAI of ~51 to ~71-75



Organism (genome accession)		Average Amino Acid Identity (AAI)									
***Bacillus subtilis* subsp. *subtilis* str. 168^T (NC_000964)**	1	1	2	3	4	5	6	7	8	9	10
Bacillus indicus LMG 22858^T (WGS:JGVU)	2	62.3									
strain SJS^T (WGS:JAVQ)	3	61.6	65.1								
***Aeribacillus pallidus* 8m3 (99.80%) (WGS:LWBR)**	4	61.1	62.9	61.7							
***Anoxybacillus pushchinoensis* K1^T (IMG:2654588147)**	5	61.6	63.4	62.2	64.3						
***Geobacillus stearothermophilus* ATCC 12980^T (WGS:JYNW)**	6	60.4	61.8	61.0	63.1	70.3					
***Caldibacillus debilis* DSM 16016^T (WGS:ARVR)**	7	57.2	57.8	57.4	59.5	60.8	60.8				
***Domibacillus robiginosus* WS 4628^T (WGS:LAHL)**	8	58.5	58.2	57.6	58.8	59.2	58.9	56.3			
***Terribacillus saccharophilus* DSM 21619^T (IMG:2636416060)**	9	56.0	54.0	54.3	54.4	56.3	55.6	53.4	54.9		
***Calditerricola satsumensis* JCM 14719^T (WGS:BBCF)**	10	54.4	55.2	54.8	55.4	57.4	58.1	55.3	53.9	52.4	
***Caldalkalibacillus thermarum* TA2.A1 (99.87%) (WGS:AFCE)**	11	53.7	54.6	54.0	55.1	57.1	56.3	53.9	52.9	51.2	58.7

Bacillus subtilis subsp. *subtilis* str. 168^T (NC_000964)	1	1	2	3	4	5	6	7	8	9	10
Bacillus subtilis subsp. *spizizenii* TU-B-10^T (NC_016047)	2	95.1									
Bacillus tequilensis KCTC 13622^T (WGS:AYTO)	3	94.0	94.4								
Bacillus vallismortis DV1-F-3^T (WGS:AFSH)	4	92.4	93.9	92.7							
Bacillus mojavensis RO-H-1^T (WGS:AFSI)	5	91.6	91.7	91.1	90.5						
Bacillus atrophaeus NBRC 15539^T (WGS:BCVV)	6	84.1	84.6	84.0	83.5	85.0					
Bacillus amyloliquefaciens DSM 7^T (NC_014551)	7	80.3	80.5	80.6	79.5	80.7	80.8				
Bacillus sonorensis NBRC 101234^T (WGS:AYTN)	8	73.4	73.1	73.1	72.3	73.3	73.4	71.7			
Bacillus glycinifermentans GO-13^T (WGS:LECW)	9	73.0	72.9	72.8	72.0	73.0	73.3	71.5	88.6		
Bacillus licheniformis DSM 13^T (NC_006270)	10	72.7	72.9	72.2	71.9	72.8	73.1	71.4	85.7	84.6	
Bacillus paralicheniformis KJ-16^T (WGS:LBMN)	11	72.6	72.8	72.2	71.6	72.6	73.0	71.6	85.4	84.5	96.0

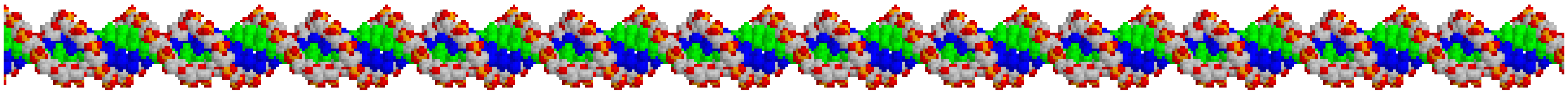
Sequence Based Comparison can ID unique and shared genes…..



Microsoft Excel - Fsucc Venn for poster.xlsx

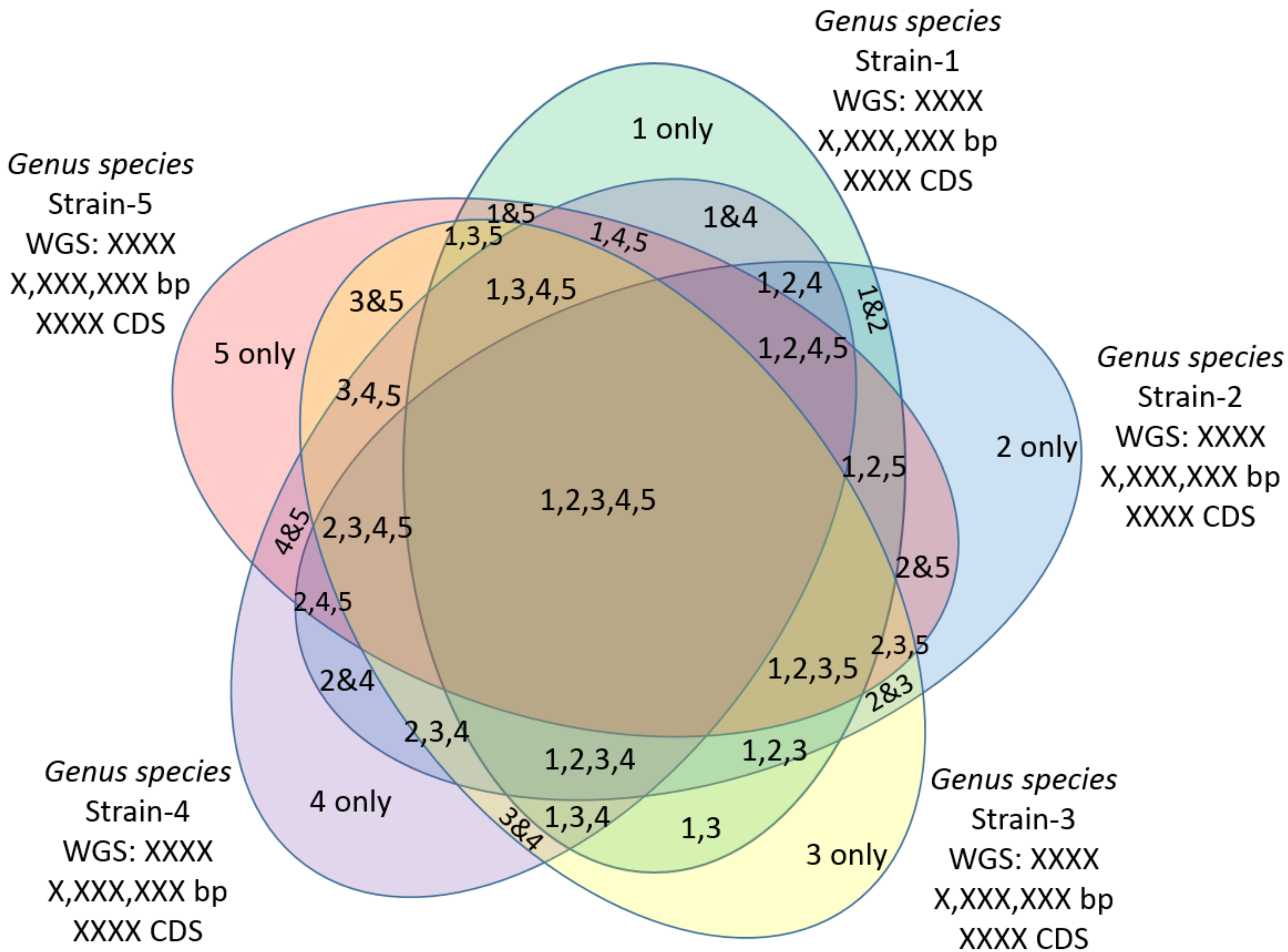
Contig	Length	Gene id	Gene	function	Hit	Contig	Gene	Gene id	percent	function	Hit	Contig	Gene	Gene id	percent	function
22	149	fig|1450525.4.pe	3118	hypothetical protein					0		bi	1	2298	fig|37668	61.9	hypothetical protein
23	162	fig|1450525.4.pe	3124	Pectate lyase (EC 4.2.2.2)					0		bi	1	2696	fig|37668	73.91	Pectate lyase (EC 4.2.2.2)
25	72	fig|1450525.4.pe	3173	Glucosamine-6-phosphate deaminase (EC 3.5.99.6)	-				0		bi	1	4817	fig|37668	91.55	Glucosamine-6-phosphate deaminase (EC 3.5.99
26	408	fig|1450525.4.pe	3177	Gluconate permease, Bsu4004 homolog	-				0		bi	1	700	fig|37668	75.68	Gluconate permease, Bsu4004 homolog
26	156	fig|1450525.4.pe	3178	Endoribonuclease L-PSP	-				0		bi	1	701	fig|37668	92.26	Endoribonuclease L-PSP
26	216	fig|1450525.4.pe	3180	4-Hydroxy-2-oxoglutarate aldolase (EC 4.1.3.16) @	-				0		bi	1	703	fig|37668	73.36	4-hydroxy-2-oxoglutarate aldolase (EC 4.1.3.16)
26	372	fig|1450525.4.pe	3181	low-specificity D-threonine aldolase	-				0		bi	1	704	fig|37668	64.42	low-specificity D-threonine aldolase
26	346	fig|1450525.4.pe	3182	Membrane dipeptidase (EC 3.4.13.19)	-				0		bi	1	705	fig|37668	87.54	Membrane dipeptidase (EC 3.4.13.19)
26	259	fig|1450525.4.pe	3183	Transcriptional repressor of the fructose operon, D	-				0		bi	1	706	fig|37668	86.77	Transcriptional repressor of the fructose operon
27	245	fig|1450525.4.pe	3188	Transcriptional regulator, AraC family	-				0		bi	1	4563	fig|37668	37.68	regulatory protein; PcrR
28	68	fig|1450525.4.pe	3197	hypothetical protein	-				0		bi	1	1909	fig|37668	36.67	hypothetical protein
29	127	fig|1450525.4.pe	3205	hypothetical protein	-				0		bi	1	2828	fig|37668	40.54	hypothetical protein
32	228	fig|1450525.4.pe	3232	hypothetical protein	-				0		bi	1	769	fig|37668	34.87	hypothetical protein
38	38	fig|1450525.4.pe	3260	Ribonucleotide reductase of class Ia (aerobic), bet	-				0		bi	1	4302	fig|37668	89.19	Ribonucleotide reductase of class Ia (aerobic), b
39	94	fig|1450525.4.pe	3261	hypothetical protein	-				0		bi	1	4967	fig|37668	61.96	hypothetical protein
1	258	fig|1450525.4.pe	38	Possible restriction endonuclease	-				0		-				0	
1	274	fig|1450525.4.pe	56	Mobile element protein	-				0		-				0	
1	484	fig|1450525.4.pe	171	Predicted transcriptional regulator containing an I	-				0		-				0	
1	232	fig|1450525.4.pe	190	Sll8048 protein	-				0		-				0	
1	1568	fig|1450525.4.pe	191	Type I restriction-modification system, M subunit	-				0		-				0	
1	317	fig|1450525.4.pe	202	COG1242: Predicted Fe-S oxidoreductase	-				0		-				0	
1	257	fig|1450525.4.pe	246	probable integral membrane protein Cj1166c	-				0		-				0	
1	163	fig|1450525.4.pe	247	FIG001826: putative inner membrane prote	-				0		-				0	
1	829	fig|1450525.4.pe	262	Succinoglycan biosynthesis protein	-				0		-				0	
2	558	fig|1450525.4.pe	303	Formate--tetrahydrofolate ligase (EC 6.3.4.3	-				0		-				0	
2	364	fig|1450525.4.pe	373	Cyanophycinase (EC 3.4.15.6)	-				0		-				0	
2	331	fig|1450525.4.pe	384	Homoserine kinase (EC 2.7.1.39)	-				0		-				0	
2	461	fig|1450525.4.pe	402	Type I restriction-modification system, specificity s	-				0		-				0	
2	76	fig|1450525.4.pe	432	Helix-turn-helix motif	-				0		-				0	
2	319	fig|1450525.4.pe	434	HipA protein	-				0		-				0	
2	68	fig|1450525.4.pe	448	DNA-binding domain of ModE	-				0		-				0	
2	172	fig|1450525.4.pe	451	Ubiquinol-cytochrome C reductase iron-sulfur sub	-				0		-				0	
2	350	fig|1450525.4.pe	454	Nitrate/nitrite transporter	-				0		-				0	
2	501	fig|1450525.4.pe	460	Cytochrome c552 precursor (EC 1.7.2.2)					0		-				0	
2	193	fig|1450525.4.pe	461	Cytochrome c nitrite reductase, small subunit NrfH	-				0		-				0	
2	79	fig|1450525.4.pe	475	B12 binding domain / kinase domain / Methylmalc	-				0		-				0	
2	119	fig|1450525.4.pe	490	Helix-turn-helix motif	-				0		-				0	

Venn Diagram Tool

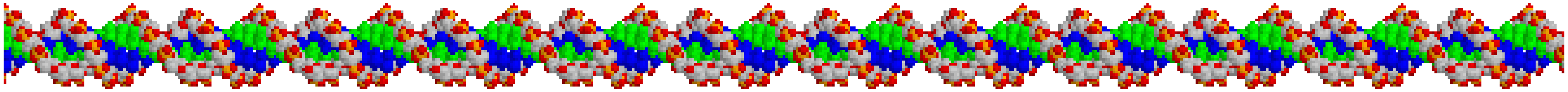


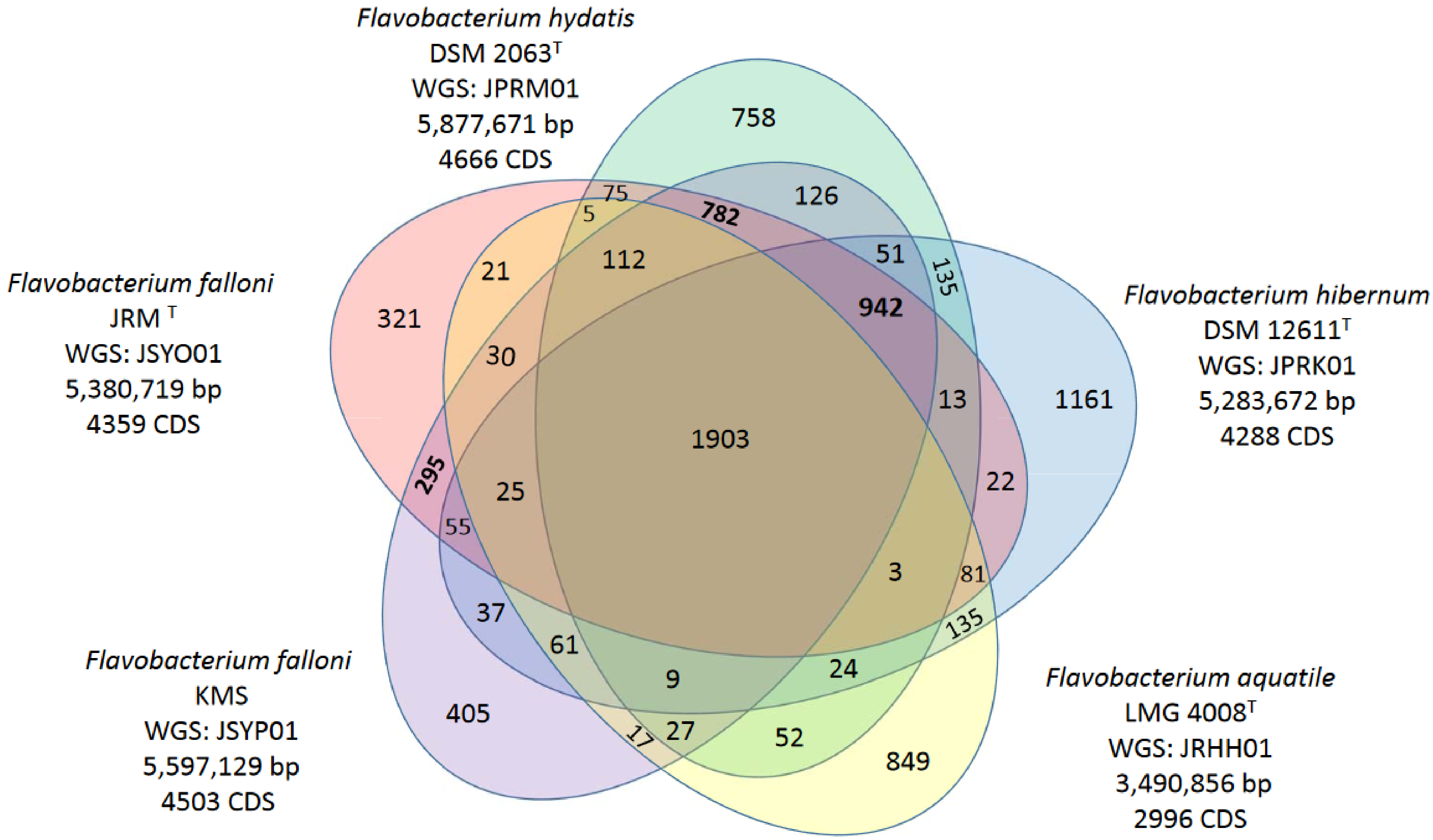
	B.cibi	B.indicus HU36	B.indicus LMG	B.colbertis SJS	B.subtilis		totals	AVG	
Only in Reference	159	523	213	1256	1656		all	2023	B.cibi, B.indicus HU36, B.indicus LMG, B.colbertis SJS, B.subtilis
Ref and All	2056	1920	2057	2047	2036		1234	633	B.cibi, B.indicus HU36, B.indicus LMG, B.colbertis SJS
1,2,3 and Ref	636	629	639	626	229		1235	237	B.cibi, B.indicus HU36, B.indicus LMG, B.subtilis
1,2,4 and Ref	239	245	236	14	13		1245	17	B.cibi, B.indicus HU36, B.colbertis SJS, B.subtilis
1,3,4 and Ref	14	26	67	73	78		1345	72	B.cibi, B.indicus LMG, B.colbertis SJS, B.subtilis
2,3,4 and Ref	69	19	3	4	9		2345	9	B.indicus HU36, B.indicus LMG, B.colbertis SJS, B.subtilis
1,2 and Ref	742	816	742	14	10		123	767	B.cibi, B.indicus HU36, B.indicus LMG
1,3 and Ref	9	20	41	56	40		124	14	B.cibi, B.indicus HU36, B.colbertis SJS
1,4 and Ref	9	17	30	11	13		125	12	B.cibi, B.indicus HU36, B.subtilis
2,3 and Ref	48	11	3	3	8		134	42	B.cibi, B.indicus LMG, B.colbertis SJS
2,4 and Ref	28	9	7	18	24		135	33	B.cibi, B.indicus LMG, B.subtilis
3,4 and Ref	11	65	15	15	13		145	12	B.cibi, B.colbertis SJS, B.subtilis
1 and ref	57	89	154	19	17		234	6	B.indicus HU36, B.indicus LMG, B.colbertis SJS
2 and ref	148	87	49	39	33		235	8	B.indicus HU36, B.indicus LMG, B.subtilis
3 and ref	17	75	24	25	15		245	36	B.indicus HU36, B.colbertis SJS, B.subtilis
4 and ref	10	61	13	224	220		345	14	B.indicus LMG, B.colbertis SJS, B.subtilis
							12	73	B.cibi and B.indicus HU36
							13	151	B.cibi and B.indicus LMG
							14	18	B.cibi and B.colbertis SJS
							15	14	B.cibi and B.subtilis
							23	68	B.indicus HU36 and B.indicus LMG
							24	57	B.indicus HU36 and B.colbertis SJS
							25	47	B.indicus HU36 and B.subtilis
							34	25	B.indicus LMG and B.colbertis SJS
							35	14	B.indicus LMG and B.subtilis
							45	222	B.colbertis SJS and B.subtilis
							1	159	B.cibi
							2	523	B.indicus HU36
							3	213	B.indicus LMG
							4	1256	B.colbertis SJS
							5	1656	B.subtilis

Venn Diagram Template

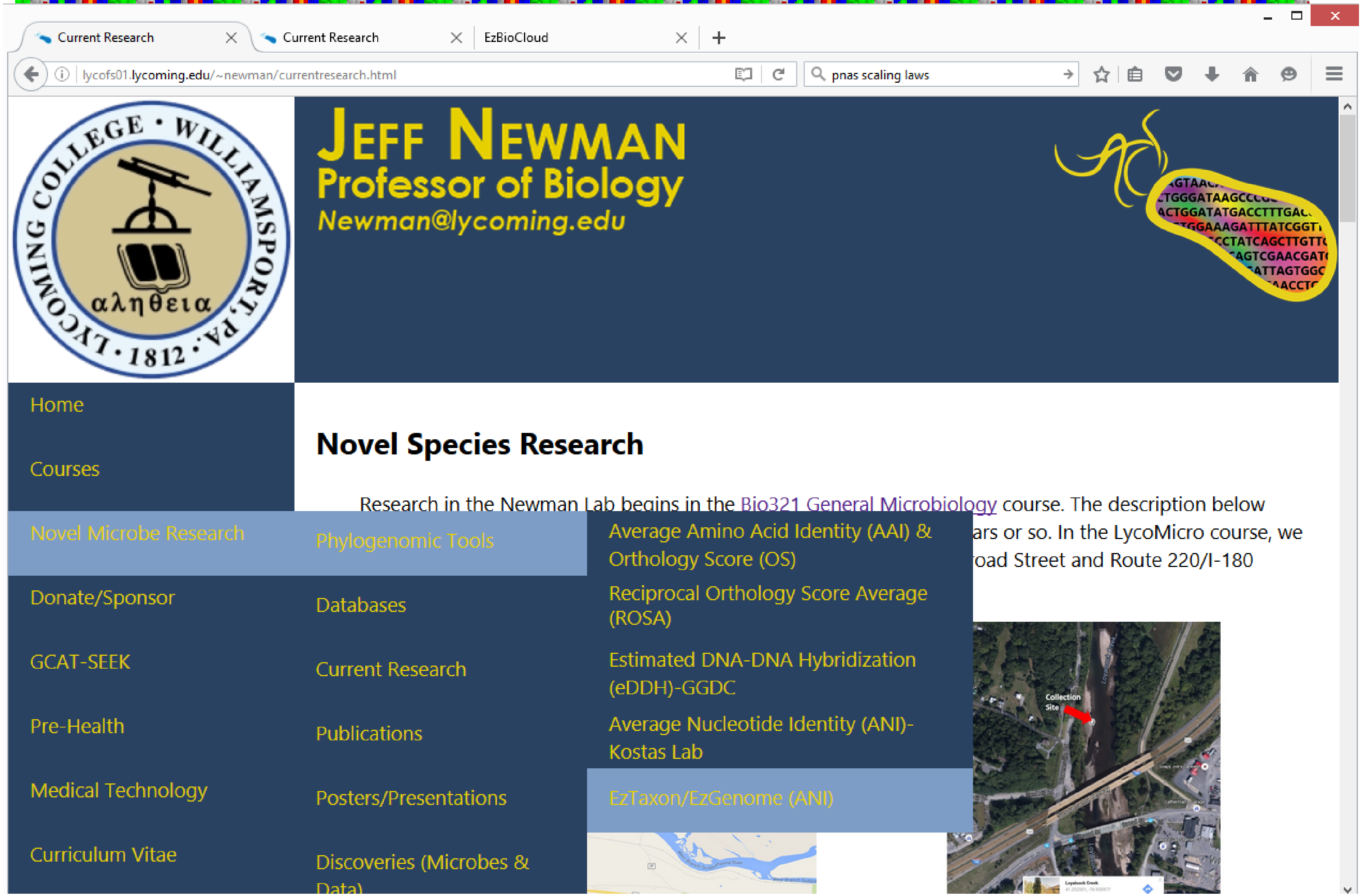


Identify Core, Genus or Family-Specific Genes



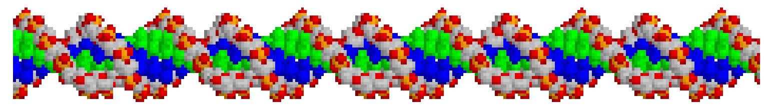


Links/Tools available at novelmicrobe.com



c and Evolutionary Microbiology (2011), **61**, 2162–2166 **DOI** 10.1099/ijs.0.027805-0

Chryseobacterium piperi sp. nov., isolated from a freshwater creek



Brittane L. Strahan,† Kevin C. Failor, Allison M. Batties,‡ Patrick S. Hayes,§ Kellie M. Cicconi,‖ Christopher T. Mason¶ and Jeffrey D. Newman

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As part of an undergraduate microbiology course, a yellow–orange
negative, rod-shaped, non-motile bacterial strain, designated CTM^T,
North-central Pennsylvania during the winter of 2006. The 16S rRNA
showed ~97 % similarity to that of *Chryseobacterium soldanellicola*
Chryseobacterium soli JS6-6^T, while the protein-coding *gyrB* gene
showed <87 % similarity to those of its two closest relatives. Using a
CTM^T was characterized and compared to these and other closely r

matic and Evolutionary Microbiology (2013), **63**, 4777–4783 **DOI** 10.1099/ijs.0.054478-0

Chryseobacterium angstadtii sp. nov., isolated from a newt tank

Karen E. Kirk,† Jessica A. Hoffman, Katherine A. Smith,‡ Brittane L. Strahan,§ Kevin C. Failor,¶ Jordan E. Krebs,‖ Andrew N. Gale, Tri D. Do, Thomas C. Sontag, Allison M. Batties,†† Kimberly Mistiszyn‡‡ and Jeffrey D. Newman

Biology Department, Lycoming College, Williamsport, PA 17701, USA

As part of an undergraduate microbiology course, a yellow–orange-pigmented, Gram-staining negative, rod-shaped, non-motile bacterial strain was isolated from a glass tank housing several red-spotted newts (*Notophthalmus viridescens*). The sequence of the 16S rRNA gene of this strain, designated KM^T, was 97.4–98.0 % similar to those of the type strains of *Chryseobacterium*

c and Evolutionary Microbiology (2014), **64**, 3804–3809 **DOI** 10.1099/ijs.0.068205-0

Genome-based reclassification of *Bacillus cibi* as a later heterotypic synonym of *Bacillus indicus* and emended description of *Bacillus indicus*

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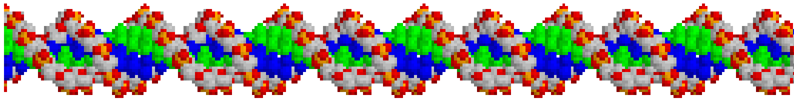
While characterizing a related strain, it was noted that there was little difference between the 16S rRNA gene sequences of *Bacillus indicus* LMG 22858^T and *Bacillus cibi* DSM 16189^T. Phenotypic characterization revealed differences only in the utilization of mannose and galactose and slight variation in pigmentation. Whole genome shotgun sequencing and comparative genomics were used to calculate established phylogenomic metrics and explain phenotypic differences. The full, genome-derived 16S rRNA gene sequences were 99.74 % similar. The average nucleotide identity (ANI) of the two strains was 98.0 %, the average amino acid identity (AAI) was 98.3 %, and the estimated DNA–DNA hybridization determined by the genome–genome distance calculator was 80.3 %. These values are higher than the species thresholds for these metrics, which are 95 %, 95 % and 70 %, respectively, suggesting that these two strains should be classified as members of the same species. We propose reclassification of *Bacillus cibi* as a later heterotypic synonym of *Bacillus indicus* and an emended description of *Bacillus*

Novel Species "Micro"-libs

Abstract

As part of an undergraduate Microbiology course, a color pigmented, Gram-staining negative, rod-shaped, non-motile bacterial strain was isolated from a freshwater creek. The sequence of the 16S rRNA gene of this strain, designated XXX^T, was 9x.x – 9x.x% similar to those of *closest two-four 16S organisms*. Comparison of whole genome shotgun sequences demonstrated genomic uniqueness with average nucleotide identity (ANI) values below xx.x%. Polyphasic characterization and comparison to these relatives revealed that strain XXX^T was similar to other *Genus* strains in that it contained MK-6 as its major respiratory quinone; produced flexirubin-type pigments, oxidase and catalase; and primarily contained the fatty acids iso-$C_{15:0}$, iso-$C_{17:1}$ *ω9c*, iso-$C_{17:0}$ 3-OH, and summed feature 3 comprising $C_{16:1}$ *ω6c*, $C_{16:1}$ *ω7c*. It differed from its closest relatives in that it blah, blah, blah. Based on the results of this study, strain XXX^T represents a novel species for which the name *Genus species* sp. nov. is proposed. The type strain is XXX^T (= DSM $xxxxx^T$ = KACC $xxxxxx^T$).

Thank You!

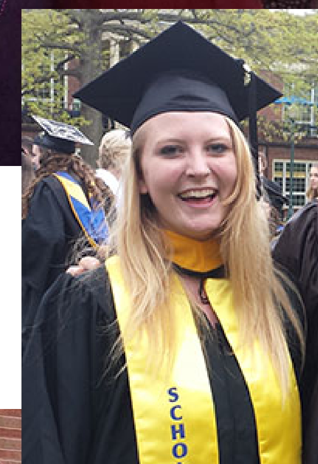


HHMI

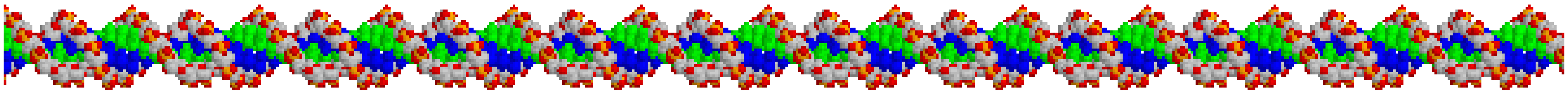
NSF

G C A T

SEEKquence



Reciprocal Orthology Score Average (ROSA)

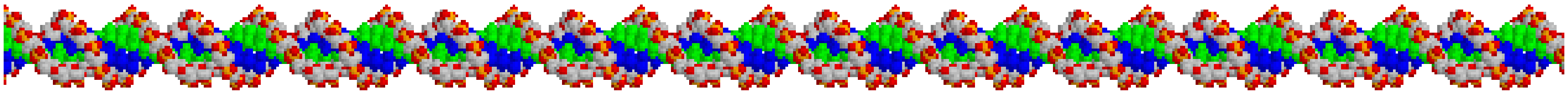


- ROSA takes into consideration both
  - similarity of orthologs at the protein level (AAI)
  - % of genome composed of orthologs (%BBH)
    - Differences in reference genome size create different %BBH values in reciprocal comparisons
- **Orthology Score (OS) = AAI^2 * %BBH**
- **ROSA = $(OS_{AB} + OS_{BA})/2$**

>~65=same species; 35-65=same genus diff species; 15-35=same family diff genus; <15=diff family

Staphylococcus (3) no type comparisons	**ROSA**	**93062.4**	**367830.3**	**93061.3**	**282459.1**	**158878.1**	**273036.3**	**282458.1**	**176279.3**	**176280.1**	**279808.3**	**342451.4**
Staphylococcus aureus subsp. aureus COL	**93062.4**											
Staphylococcus aureus subsp. aureus USA300	**367830.3**	94.8										
Staphylococcus aureus subsp. aureus NCTC 8325	**93061.3**	93.97	92.73									
Staphylococcus aureus subsp. aureus MSSA476	**282459.1**	90.39	90.1	90.85								
Staphylococcus aureus subsp. aureus Mu50	**158878.1**	88.01	88.25	88.15	87.5							
Staphylococcus aureus RF122	**273036.3**	86.78	85.6	86.89	86.02	86.18						
Staphylococcus aureus subsp. aureus MRSA252	**282458.1**	85.95	86.56	85.23	87.8	84.86	83.67					
Staphylococcus epidermidis RP62A	**176279.3**	43.49	43.38	42.5	43.55	43.37	43.55	43.97				
Staphylococcus epidermidis ATCC 12228	**176280.1**	43.25	43.25	42.62	43.7	42.69	43.71	43.22	86.44			
Staphylococcus haemolyticus JCSC1435	**279808.3**	41	41.28	40.73	41.15	40.63	41.32	41.24	44.94	44.9		
Staphylococcus saprophyticus ATCC 15305^T	**342451.4**	38.35	38.33	38.01	38.62	37.65	38.63	38.35	39.6	39.79	40.18	

Reciprocal Orthology Score Average (ROSA)



Newman@lycoming.edu

Newman Lab ROSA Calculator

INSTRUCTIONS:
1) "Export file" from RAST Sequence Comparison Tool output for up to and including 11 files.
2) Browse for the files below. Holding the CTRL key down will allow you to select all the files at once by clicking on each of them; or click on the first file, then hold down the SHIFT key and click on the last file to select the range.
3) Hit the "Submit" button.
4) Copy and Paste Results Table to a Separate Spreadsheet or Word Processor Document.

Browse... 11 files selected.

Submit

Sample .tsv files(must be unzipped)

A calculator to determine the Orthology Score (OS) from a single .tsv file is available here.
When using this website or the Perl script for your study, please cite the following

hold down the SHIFT key and click on the last file to select the
3) Hit the "Submit" button.
4) Copy and Paste Results Table to a Separate Spreadsheet
Document.

Browse... 11 files selected.

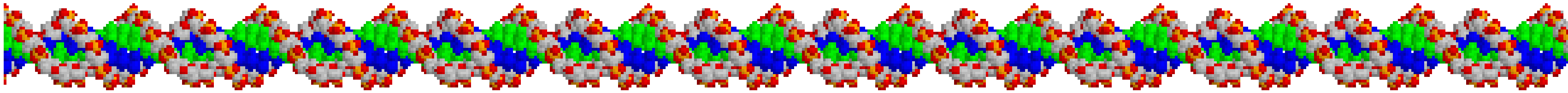
Submit

ROSA Analysis

Orthology Analysis for Each Reference Genome

	Genome ID	AAIr	% BBH	OS
ref	1177926.4			
1	293387.12	98.390	93.751	90.756
2	1049581.5	98.760	93.244	90.946
3	1664069.5	68.720	76.416	36.087
4	1648923.6	68.482	77.028	36.124
5	536229.12	93.299	90.624	78.885
6	1355022.6	93.427	91.458	79.831
7	1220591.5	68.694	75.730	35.736
8	1236481.12	98.608	91.937	89.395
9	224308.127	69.187	76.003	36.381
10	1178537.4	94.663	87.872	78.742

ROSA Thresholds



Level	Same	Different	expected range	min	max	mean	n=	below range	above range
8	species	strain	>65	49	99.59	85.98	312	3	N/A
7	genus	species	35-65	6.85	95.8	36.63	521	294	38
6	family	genus	15-35	5.8	54.99	18.86	524	125	19
5	order	family	10-15	4.75	23.19	11.58	314	79	22
4	class	order	8-10	4.15	14.99	8.22	286	121	58
3	phylum	class	6-8	4.3	11.4	6.62	123	42	14
2	domain	phylum	3-6	1.85	7.66	4.45	211	10	6
1		domain	<3	1.36	4.44	2.42	47	0	9

Table 4. ROSA Values at Different Phylogenetic levels.