

Gall-ID: web-based tools for the rapid identification and characterization of gall-causing phytopathogenic bacteria

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Abstract

Understanding the genetic diversity of plant pathogens and the effect of agricultural practices on pathogen evolution are important for disease management. Advances in DNA sequencing technology have contributed to greater reliance on use of 16S rDNA, multilocus sequence analysis (MLSA), and whole genome sequences to genotype bacteria. Correct analysis and interpretation of sequencing data can be difficult. Therefore we have developed a set of web-based tools, termed Gall-ID, to facilitate the identification and characterization of phytopathogenic bacteria, with a focus on those that cause gall diseases. Users can compare 16S or MLSA gene sequences from an isolate against manually-curated databases, and generate a phylogenetic tree containing their isolate. Gall-ID also includes a tool for uploading and using whole genome sequencing reads to identify homologs of known virulence genes. Finally, Gall-ID provides downloadable software pipelines for core genome analysis (WGS Pipeline), calculation of average nucleotide identity (Auto ANI), and the generation of MLSA gene set databases (Auto MLSA).

Introduction to Gall-ID

- Web-based tools to easily identify a bacterial isolate using 16S or MLSA gene sequences
- Enables rapid identification without bioinformatics experience
- Downloadable software tools available for core genome and whole genome comparisons
- Available at:

<http://gall-id.cgrb.oregonstate.edu>



Instructions
Select an MLSA dataset, input corresponding FASTA format DNA sequences for one isolate, and select submit. Copy and paste your DNA sequences in FASTA format into the window below. Sequences should be named by gene name (ie ">gyrB"). Not all genes are required to be input, though accuracy will be improved with a complete set of MLSA gene sequences. Use the options in the Analysis section to generate a Distance Tree or Minimum Spanning Network after submitting your data. These trees can be exported as pdf files or in Newick format.

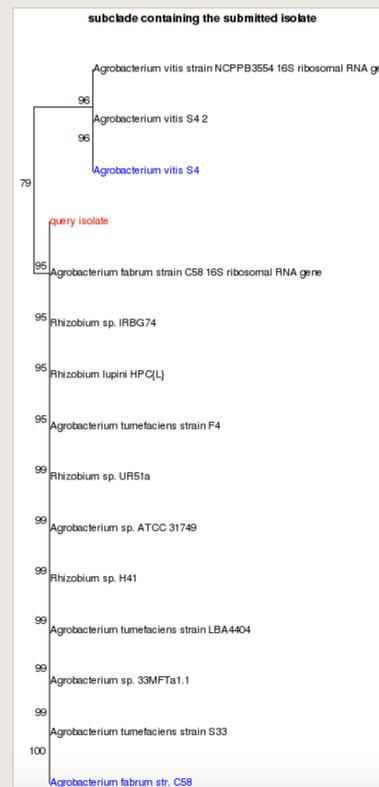
Select Dataset: Choose an MLSA or 16S gene set to compare your input sequences to:
Agrobacterium 16S

FASTA Input

```
>16S
AACGAACGCTGGCCGAGGCTTAACACATGCAAGTCAAGCCGACGAGGAGGAGTGGAGAGCGGTGATGACGCGTGGGAATCTACCCA
TCTCTGGGAAATAGCTCTGGGAACTGGAATTAATACCGCATACGCCCTACGGGGAAGATTTATCGGGATGGATGAGCCGGCTGGTGGT
AGCTAGTGGTGGGTAAGGCTACCAAGGGGACGATCATAGCTGGTCTGAGAGAGATGATCAGCCACATTTGGGACTGAGACACGGCCAA
ACTCTACGGGAGGCGAGTGGGGAATTTGGACAATGGGCGAAGCGCTGATCCAGCCATGCGCGTGGATGATGAGGCGCTAGGGTGT
AAAGCTCTTTCAGCGATAGAGATAATGACGGTACGTGCGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
AGCGTTGTGGGAATTTACGGGCTAAAGCCGACGTAGGCGGATATTAAAGTCAAGGGGTAATCCGAGCTCACTGGGAGCTGCTCTT
ATACCTGGATCTTGAGTATGGAAGAGTAAAGTGGATTCCGAGTGTAGAGGTGAATTCGATATTGGGGAACACCGATGGGGAAGCG
GCTTACTGTGCTCATTAGCAGCTGAGGTGCGAAGCGTGGGAGGAGCAACAGGATTAGATACCCCTGGTGGTCCAGCGGTAACGATGAAT
TAGCCGTGGGCGATGATCTGTTGGTGGGCGCAAGCTAAACATTCGCGCTGGGAGTACGGTCCGCAAGATTAACCTCAAGGAAT
TGACGGGGGCGCGCACAGCGGGTGGAGCATGTGTTAATTCGAGAGAACGCGCAGACCTTACAGCTCTTGACACTCGGGGTATGGCGATT
GGAGACGATGCTTCAAGTAAAGCTGGGCGCACAGCGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT
CGAGCGAACCTCCGCTTGGTGGCAGCATTAAGTGGGCACTTAAGGGGACTGCCGGTATAGAGCGAGAGAGAGAGAGAGAGAGAGAG
CAAGCTCATGGCCCTACGGGCGGTGACACAGCTGCTCAATGTTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT
AAAGCCATCTCAGTTCGATGCTGCAACTCGAGTGGATGAAGTGGATGCTAGTAAATCGATGATCGATGATCGATGATCGATGATCGATG
```

Random Seed: Choose a Distance: Kimura 80

Select an organism, a 16S or MLSA gene dataset, and input your sequences



Gall-ID produces and displays a phylogenetic tree containing your isolate for easy identification

16S database

MLSA databases:

- *Agrobacterium*
- *Clavibacter*
- *Dickeya*
- *Pantoea agglomerans*
- *Pectobacterium*
- *Pseudomonas savastanoi*
- *Ralstonia*
- *Rhodococcus fascians*
- *Xanthomonas*
- *Xylella*

Identify the presence of known virulence genes in raw whole genome sequencing data: Gene coverage and read-mapping depth as well as the most closely related organism allele for each gene are reported.

Example output of the Vir-Search tool

Virulence Gene Search results

Submitted job name: test16
Organism: Agrobacterium
Forward/Single reads file: A1_1258.trimmed.1.fastq
Reverse reads file:
Minimum % coverage of database gene: 90%
Maximum % divergence from database gene: 10%
Output file prefix: 141183284_822636_141183284_822636_out_16genes_agrovib_results.txt

Result table

Agrobacterium tumefaciens CS8 genes used as reference for all virulence genes except for GALLS which came from Agrobacterium tumefaciens strain CS8

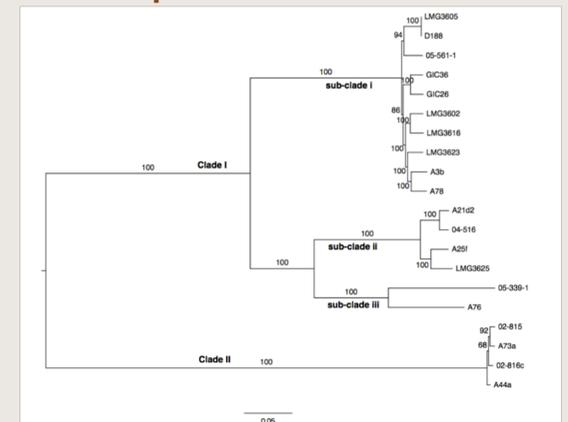
Found	Gene	Coverage (%)	Depth	Closest Allele
+	tms2	100.0	31.699	Rhizobium_rubi_NBRC_13261
+	tms1	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	ipt	100.0	36.349	Rhizobium_rubi_NBRC_13261
x	galls	-	-	-

Oncogenes

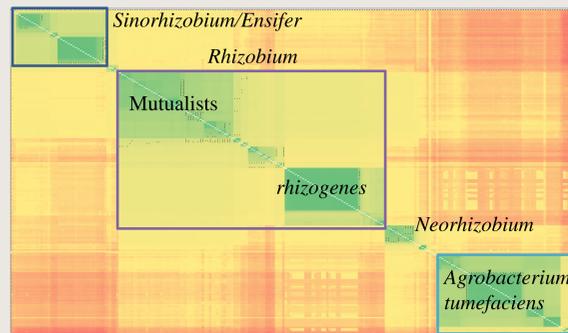
Found	Gene	Coverage (%)	Depth	Closest Allele
+	vrs0	100.0	28.21	Rhizobium_rubi_NBRC_13261
+	vrs4	100.0	30.795	Rhizobium_rubi_NBRC_13261
+	vrs5	100.0	23.916	Agrobacterium_arsenivivans_strain_KF8_333
+	vrs6	100.0	31.887	Agrobacterium_arsenivivans_strain_KF8_333
+	vrs7	100.0	28.272	Agrobacterium_arsenivivans_strain_KF8_333
+	vrs8	100.0	35.159	Rhizobium_rubi_NBRC_13261
+	vrs9	100.0	27.213	Rhizobium_rubi_NBRC_13261
+	vrs10	100.0	27.482	Rhizobium_rubi_NBRC_13261
+	hml1	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml2	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml3	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml4	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml5	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml6	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml7	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml8	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml9	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml10	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml11	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml12	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml13	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml14	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml15	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml16	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml17	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml18	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml19	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml20	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml21	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml22	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml23	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml24	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml25	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml26	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml27	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml28	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml29	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml30	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml31	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml32	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml33	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml34	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml35	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml36	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml37	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml38	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml39	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml40	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml41	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml42	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml43	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml44	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml45	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml46	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml47	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml48	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml49	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	hml50	100.0	27.473	Rhizobium_rubi_NBRC_13261

Simplified whole genome analysis: Use whole genome sequencing reads and reference genome sequences to generate a core genome phylogeny, or calculate pairwise average nucleotide identity (ANI) between genome assemblies, with minimal user input.

Phylogenetic tree of *R. fascians* genomes generated using the WGS Pipeline



Average nucleotide identity (ANI) heatmap of *A. tumefaciens* genomes generated using Auto ANI



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