

Finding genes associated with antibiotic resistance in *Pseudomonas* and *Klebsiella*

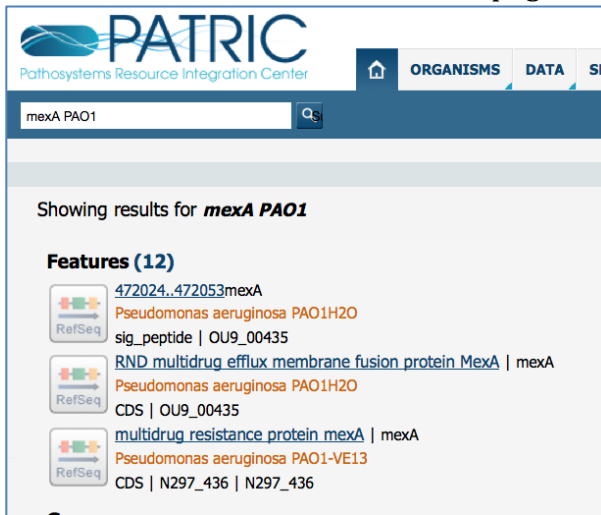
This exercise demonstrates two different ways to look for antibiotic resistance genes in PATRIC. The first is to do a direct search with the gene name and a popular *Pseudomonas* strain. The second is to use PATRIC's specialty gene search. We will begin with the *mexA* gene in *Pseudomonas aeruginosa* strain PAO1. This strain of *P. aeruginosa* is one of NCBI's reference strains and PATRIC has incorporated 46 transcriptomic experiments for this genome.

I. Searching for a specific gene in a specific strain of *Pseudomonas*

1. Search for the gene name (*mexA*) and the strain (PAO1) in the global search box on PATRIC and hit return.



2. This will open up the search results page. You can see that there are 12 features that match the search criteria. This page shows the top three results.













3. To see all the results, click on the number that follows the word "Features."



4. This will display all the genes that match the search criteria.

Showing 12 feature(s) for: **mexA PAO1**

Select all (12) displayed feature(s) Show Toolbar

<input type="checkbox"/>		472024..472053 mexA Pseudomonas aeruginosa PAO1H20 sig_peptide OU9_00435
<input type="checkbox"/>		RND multidrug efflux membrane fusion protein MexA mexA Pseudomonas aeruginosa PAO1H20 CDS OU9_00435
<input type="checkbox"/>		multidrug resistance protein mexA mexA Pseudomonas aeruginosa PAO1-VE13 CDS N297_436 N297_436
<input type="checkbox"/>		multidrug resistance protein mexA mexA Pseudomonas aeruginosa PAO1-VE2 CDS N296_436 N296_436
<input type="checkbox"/>		RND multidrug efflux membrane fusion protein MexA precursor mexA Pseudomonas aeruginosa PAO1 CDS PA0425 PA0425
<input type="checkbox"/>		multidrug resistance protein mexA mexA Pseudomonas aeruginosa PAO581 CDS M801_436 M801_436
<input type="checkbox"/>		multidrug resistance protein mexA Pseudomonas aeruginosa PAO1-GFP CDS V563_05235
<input type="checkbox"/>		RND efflux system, membrane fusion protein CmeA mexA Pseudomonas aeruginosa PAO1-VE2 CDS fig 1367493.3.peg.446 N296_436 VBIPseAer310614_0446
<input type="checkbox"/>		RND efflux system, membrane fusion protein CmeA mexA Pseudomonas aeruginosa PAO1-VE13 CDS fig 1367494.3.peg.446 N297_436 VBIPseAer305084_0446
<input type="checkbox"/>		RND efflux system, membrane fusion protein CmeA mexA Pseudomonas aeruginosa PAO1H20

5. To find the gene in the PAO1 strain, click on the blue product description (RND multidrug efflux membrane fusion protein MexA precursor).

<input type="checkbox"/>		RND multidrug efflux membrane fusion protein MexA precursor mexA Pseudomonas aeruginosa PAO1 CDS PA0425 PA0425
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6. This will take you to the landing page for the mexA gene in the PAO1 strain. Look carefully at the page. It includes summary information across the very top, tabs across the top of the page just above the tables, links to different information along the left-hand side, and boxes with summary information.

Bacteria > Proteobacteria > Gammaproteobacteria > Pseudomonadales > Pseudomonadaceae > Pseudomonas > Pseudomonas aeruginosa PAO1 > fig | 208964.12.peg.447 | PA0425 | VBIpseAer58763_0447 | mexA | RND efflux system, membrane fusion protein CmeA

Overview Genome Browser Compare Region Viewer Pathways Transcriptomics Interactions Correlated Genes Literature

Add PATRIC Feature to Workspace

View NT Sequence
View AA Sequence

External Tools

The SEED Viewer
NCBI CDD Search
STRING: Protein-Protein Interactions
STITCH: Chemical-Protein Interactions

Recent PubMed Articles

- 2015 Apr 28
Expression of the mexA Gene Requires the DNA Helicase RecG in Pseudomonas aeruginosa PAO1. Heo A and Park W J Microbiol Biotechnol
- 2015 Apr
Reduced production of OprM may promote oprD mutations and lead to imipenem resistance in Pseudomonas aeruginosa carrying an oprD-group

Gene ID: PATRIC ID: fig|208964.12.peg.447 RefSeq: PA0425 Alt Locus Tag: VBIpseAer58763_0447

Protein ID: RefSeq: NP_249116.1 UnitProt: P52477 80 IDs are mapped

mexA

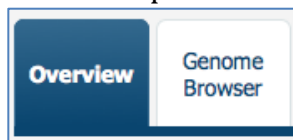
CDS

Annotation	Locus Tag	Start	End	NT Length	AA Length	Product
PATRIC	VBIpseAer58763_0447	472042	473175	1134	377	RND efflux system, membrane fusion protein CmeA
RefSeq	PA0425	472024	473175	1152	383	RND multidrug efflux membrane fusion protein MexA precursor

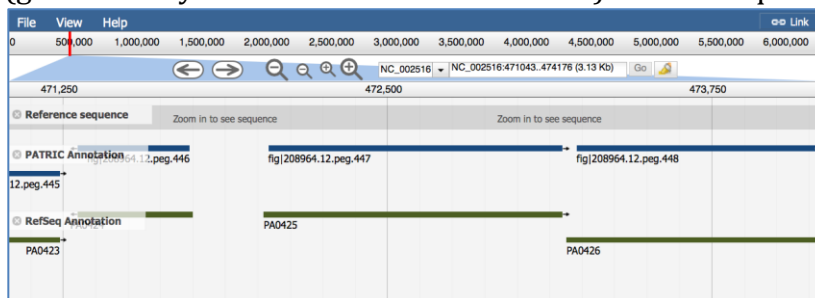
Functional Properties

GO Assignments	-
EC Assignments	-
FIGfam Assignments	FIG01304998
Pathway Assignments	-
Structure	Not supported by SSGCID/CSGID
Protein Interactions	Host-pathogen interactions: 0 All interactions: 0

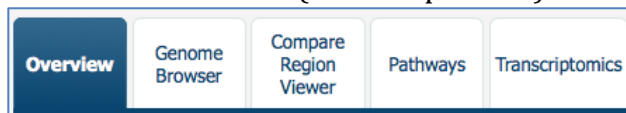
7. We'll explore the tabs for this gene. Click on the second tab (Genome Browser).



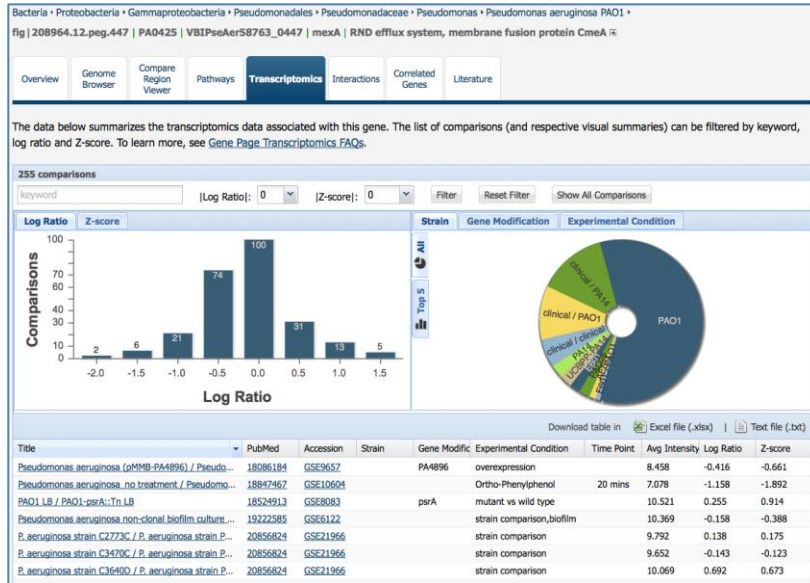
8. This will open up the genome browser, which shows the PATRIC annotation (generated by RAST and with the blue lines) and RefSeq annotation (green lines).



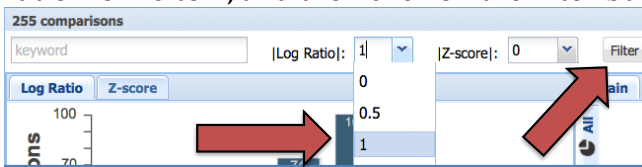
9. Click on the 5th tab (Transcriptomics)



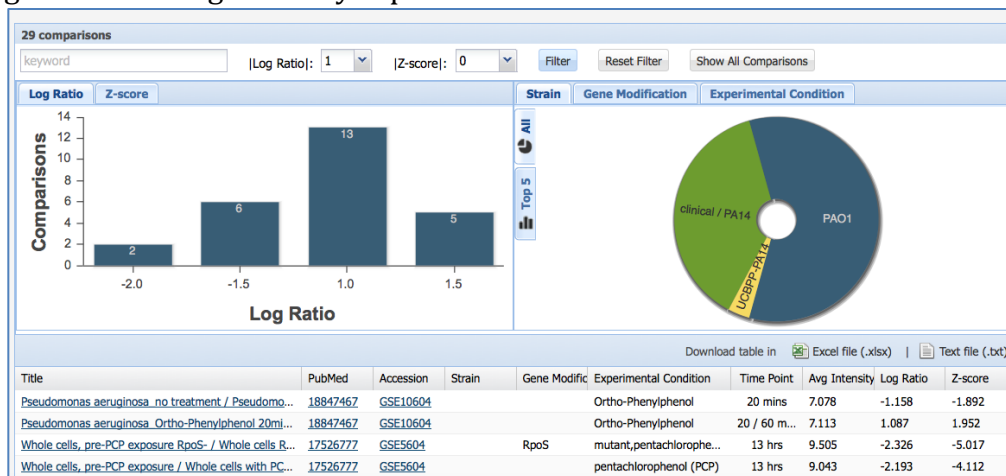
10. This is where you will find a summary of all the transcriptomics experimental evidence we have in PATRIC for this particular gene. The top of the page has a filter that allows you to search for specific results. Below are two summary graphs that display the information, followed by a table with the title and other information on the experiment, including the log ratio and Z score for the gene in that particular experiment.



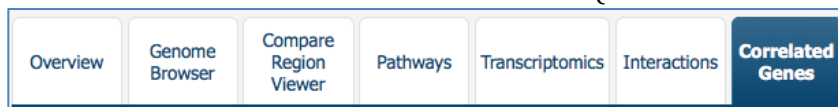
11. Along the top, you can filter on different levels of expression. Change the log ratio from 0 to 1, and then click on the filter button.



12. The results of the filter will show you the experimental conditions where your gene matches that value, and also shows the experimental conditions where the gene is more significantly expressed.



13. Return to the tabs and click the 7th one (Correlated Genes)



18. This will open the page that shows correlated expression with the mexA gene. The top row contains the mexA gene itself. The second row, with the highest correlation to the mexA gene is mexB.

Correlation Cutoff: 0.4 Correlation: positive Filter

10 features found

Genome Name	PATRIC ID	Alt Locus Tag	RefSeq Locus Tag	Gene Symbol	Product Description	Correlation
Pseudomonas aeruginosa PAO1	fig 208964.12.peg.447	VBIPseAer58763_0447	PA0425	mexA	RND efflux system, membrane fusion protein CmeA	1.000
Pseudomonas aeruginosa PAO1	fig 208964.12.peg.448	VBIPseAer58763_0448	PA0426	mexB	RND efflux system, inner membrane transporter C...	0.581
Pseudomonas aeruginosa PAO1	fig 208964.12.peg.3056	VBIPseAer58763_3056	PA2915		Zn-dependent hydrolases, including glyoxylases	0.470
Pseudomonas aeruginosa PAO1	fig 208964.12.peg.2958	VBIPseAer58763_2958	PA2821		Glutathione S-transferase family protein	0.442
Pseudomonas aeruginosa PAO1	fig 208964.12.peg.449	VBIPseAer58763_0449	PA0427	oprM	RND efflux system, outer membrane lipoprotein Cm...	0.436
Pseudomonas aeruginosa PAO1	fig 208964.12.peg.106	VBIPseAer58763_0106	PA0102		Carbonic anhydrase (EC 4.2.1.1)	0.420
Pseudomonas aeruginosa PAO1	fig 208964.12.peg.5260	VBIPseAer58763_5260	PA5018	msrA	Peptide methionine sulfoxide reductase MsrA (EC 1...	0.406
Pseudomonas aeruginosa PAO1	fig 208964.12.peg.58	VBIPseAer58763_0058	PA0055		Uncharacterized protein conserved in bacteria	0.406
Pseudomonas aeruginosa PAO1	fig 208964.12.peg.3262	VBIPseAer58763_3262	PA3110	DedD	DedD protein	0.400
Pseudomonas aeruginosa PAO1	fig 208964.12.peg.3204	VBIPseAer58763_3204	PA3053		2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrol...	0.400

19. Go back to the 1st tab (Overview).

Bacteria > Proteobacteria > Gammaproteobacter

fig | 208964.12.peg.447 | PA0425 | VBIPs

Overview Genome Browser Compare Region Viewer Path

Add PATRIC Feature to Workspace

View NT Sequence

View AA Sequence

External Tools

The SEED Viewer

NCBI CDD Search

STRING: Protein-Protein Interactions

STITCH: Chemical-Protein Interactions

20. Along the upper left hand side, under “External Tools” there are a number of hyperlinks to other resources that have information about the mexA gene. These include SEED, Conserved Domain Database (CDD), String and Stitch. Clicking on any of the title will open a new tab on your browser to the other resources, and will land on the mexA gene there.

Functional Properties	
GO Assignments	-
EC Assignments	-
FIGfam Assignments	FIG01304998
Pathway Assignments	-
Structure	Not supported by SSGCID/CSGID
Protein Interactions	Host-pathogen interactions: 0 All interactions: 0

23. This will take you to a table that shows all the members of the protein family that contains mexA, and includes a large number of the genomes currently in PATRIC.

Protein Family Members

Keyword: Filter Table

19607 features found in FIG01304998

Workspace

Add Feature(s)

View

FASTA DNA

FASTA Protein

Download

Table

FASTA

Tools

Pathway Summary

MAP IDs to...

Columns

Show/Hide

Default

<input type="checkbox"/>	Genome Name	PATRIC ID	RefSeq Locus Tag	Alt Locus Tag	Gene Symbol
<input type="checkbox"/>	Acinetobacter baumannii 470922	fig 1310808.3.peg.2326	J707_2400	VBIACiBau281795_2326	
<input type="checkbox"/>	Enterobacter sp. B509	fig 137719.3.peg.3893		VBIEntSp64224_3893	
<input type="checkbox"/>	Acinetobacter nosocomialis 28F	fig 1147131.5.peg.1506		VBIACiNos231591_1506	
<input type="checkbox"/>	Xanthomonas axonopodis pv. manihotis str. IBSBF 2538	fig 1185675.3.peg.72		VBIxanAxo245698_0072	
<input type="checkbox"/>	Delftia sp. 670	fig 1537702.3.peg.27	GY15_00115		
<input type="checkbox"/>	Salmonella enterica subsp. enterica serovar Choleraesuis ...	fig 321314.9.peg.3877		VBISalEnt136302_3877	
<input type="checkbox"/>	Salmonella enterica subsp. enterica serovar Choleraesuis ...	fig 904139.4.peg.2130		VBISalEnt174419_2130	
<input type="checkbox"/>	Salmonella enterica subsp. enterica serovar Choleraesuis ...	fig 997330.3.peg.2430		VBISalEnt302986_2430	
<input type="checkbox"/>	Salmonella enterica subsp. enterica serovar Choleraesuis ...	fig 938142.3.peg.3456		VBISalEnt312252_3456	

24. If you wanted to find if any Klebsiella genomes contained mexA genes in this same protein family you could enter “Klebsiella” in the Keyword search at the top of the table and click on the “Filter Table” button.

Protein Family Members

Keyword: Filter Table

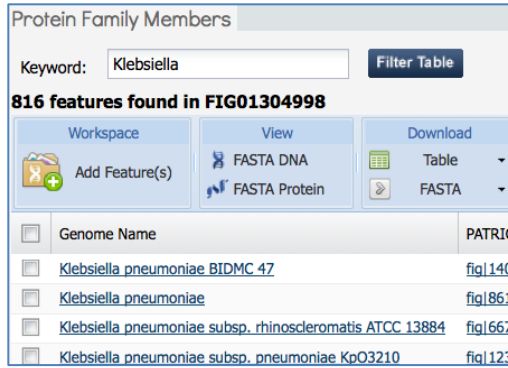
19607 features found in FIG01304998

Workspace

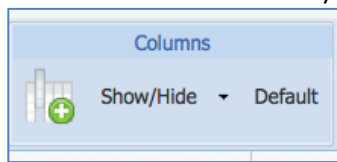
View

Download

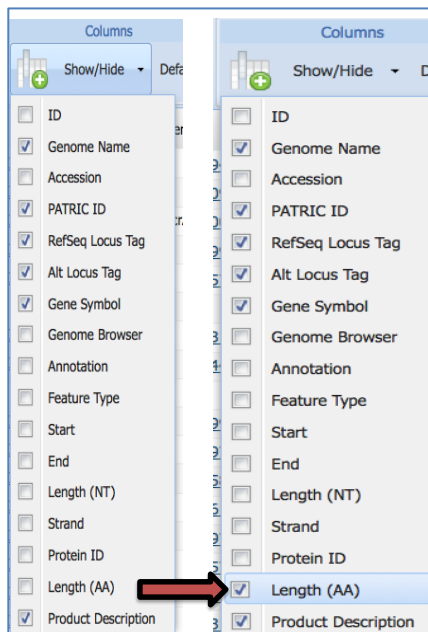
25. This will show you the number of genes across all the Klebsiella genomes that are members of this family. Note that the number along the top of the table has changed.



26. You can add a column to show additional information, by clicking on the down arrow next to the Show/Hide icon.



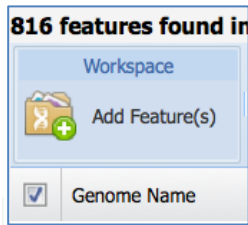
30. Click on the box in front of Length (AA) to see the length of the protein, measured in amino acids.



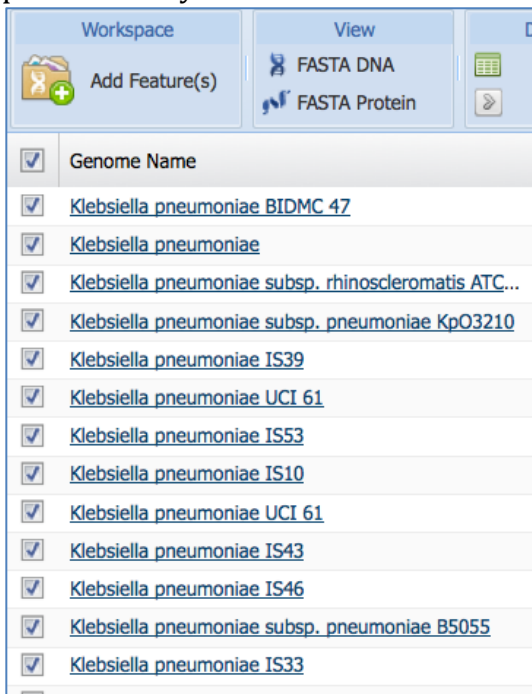
31. This will open up a new column that shows the lengths of the Klebsiella proteins that are included in this family.

Length (AA)	Product Description
68	RND efflux system, membrane fusion protein CmeA
74	RND efflux system, membrane fusion protein CmeA
74	RND efflux system, membrane fusion protein CmeA
138	RND efflux system, membrane fusion protein CmeA
177	RND efflux system, membrane fusion protein CmeA
177	RND efflux system, membrane fusion protein CmeA
187	RND efflux system, membrane fusion protein CmeA
187	RND efflux system, membrane fusion protein CmeA
210	RND efflux system, membrane fusion protein CmeA
214	RND efflux system, membrane fusion protein CmeA
214	RND efflux system, membrane fusion protein CmeA

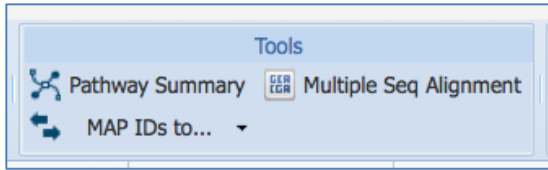
30. You can generate a multiple sequence alignment to see similarities and differences in these proteins by first clicking on the box right in front of the column "Genome Name."



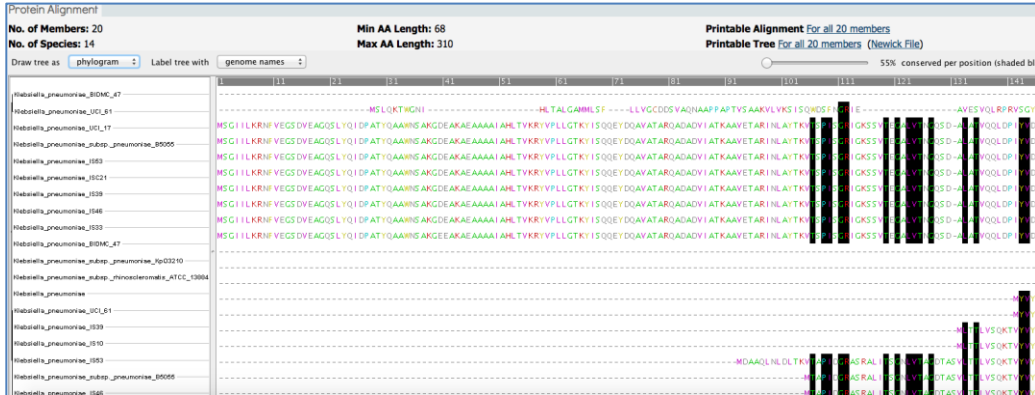
32. This will select all of the 20 genes that you see in the table that contains the protein family members.



33. In the Tools section above the table, click on the icon for “Multiple Sequence Alignment.”



34. This will open up a new window that contains an alignment (right side) and a gene tree (left side) for those first 20 proteins.



II. Looking more broadly at antibiotic resistance genes-Specialty Gene landing page for *Pseudomonas*

1. Enter Pseudomonas into the text box for the global search and hit return



2. This will open up the search results page that divides the results into Features (aka genes), Genomes, Taxonomy and Experiments.

Showing results for ***Pseudomonas***

Features (9641193)

- [630277..631365](#)
Pseudomonas alkylphenolia
CDS
- [765297..765713](#)
Pseudomonas mendocina
misc_feature
- [1135319..1135529](#)
Pseudomonas mendocina
misc_binding

Genomes (1153)

- [Pseudomonas syringae pv. aesculi str. 2250](#)
266 Contigs
SEQUENCED: 2009-08-21T00:00:00Z (The Saint
COMMENT: Pseudomonas syringae pv. aesculi s
pathogen of Horse Chestnut trees in UK.
- [Pseudomonas fluorescens Wayne1](#)
240 Contigs
SEQUENCED: 2011-06-22T00:00:00Z (The Ohio
COMMENT: -
- [Pseudomonas aeruginosa JD304](#)
1613 Contigs

Taxonomy (19436)

- [Pseudomonas \(genus\)](#)
1143 Genome(s)
- [Pseudomonas pavonaceae \(species\)](#)
0 Genome(s)
- [Pseudomonas cruciviae \(species\)](#)
0 Genome(s)

3. Scroll down to the Taxonomy results and click on the name “Pseudomonas.”

Taxonomy (19436)

[Pseudomonas \(genus\)](#)
[1143 Genome\(s\)](#)

4. This will take you to the landing page for the genus *Pseudomonas*.

Bacteria • Proteobacteria • Gammaproteobacteria • Pseudomonadales • Pseudomonadaceae • **Pseudomonas** ⓘ Data Overview Tutorial »
Download genome data »

Overview Taxonomy Phylogeny Genome List Feature Table Speciality Genes Protein Families Pathways Transcriptomics Interactions Diseases Literature

Search Tools Taxonomy Summary

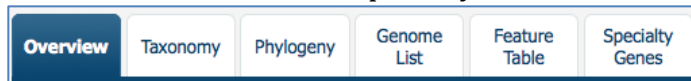
- [Genome Finder](#)
- [Feature Finder](#)
- [Comparative Pathway Tool](#)
- [Protein Family Sorter](#)

Experiment Summary

Transcriptomics from GEO	349
Transcriptomics from ArrayExpress	426
Proteomics from PRIDE	0
Structure from NCBI	2277

Taxonomy ID	286
Lineage	cellular_organisms > Bacteria > Proteobacteria > Gammaproteobacteria > Pseudomonadales > Pseudomonadaceae > Pseudomonas
External Links	Immune Epitope Database and Analysis Resource
Summary Terms - Click on number to view genomes associated with term (see PATRIC FAQs)	
Genome Status	WGS (1049), Complete (94)
Reference Genome	Representative (30), Reference (1)
Antimicrobial Resistance	Susceptible (125), Resistant (36), show all 182 genomes
Antimicrobial Resistance Evidence	AMR Panel (125)
Isolation Country	USA (259), France (79), show all 845 genomes

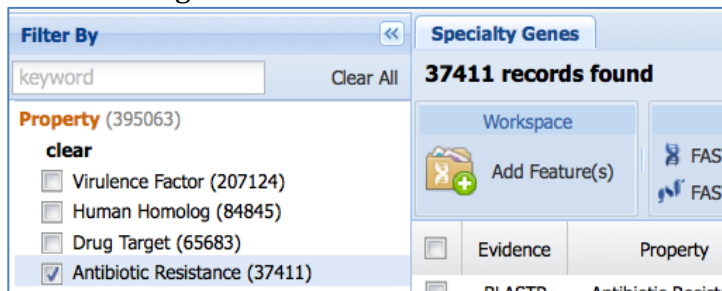
5. Click on the tab called “Specialty Genes.”



6. This will take you to the Specialty Genes landing page for Pseudomonas. On the left side there is a dynamic filter, and on the right a table that shows the genes. The table shows if the evidence came from literature or BLAST, the type of property associated with the gene (i.e. virulence, antibiotic resistance, human homolog, or drug target), various locus tags, the gene that has the actual evidence associated with it, the gene name, the product description, a link to the PubMed reference (if there is one), the percent identity of the Pseudomonas gene to the gene with the evidence, and the E-value from the BLAST hit.

Evidence	Property	Source	Genome Name	PATRIC ID	RefSeq Loc	Alt Locus Tag	Source ID	Gene	Product	Pubmed	Identity	E-value
BLASTP	Virulence Factor	VFDB	Pseudomonas aeru...	fig1983918.3.peg.5724	PA13_2...	VBIPseAer186653_5...	VFG0114		Leader peptidase (Pr...	99	0.0	
BLASTP	Virulence Factor	Victors	Pseudomonas aeru...	fig1983918.3.peg.5774	PA13_2...	VBIPseAer186653_5...	4208		Leader peptidase (Pr...	PubMed	99	0.0
BLASTP	Virulence Factor	VFDB	Pseudomonas aeru...	fig1983918.3.peg.2405	PA13_1...	VBIPseAer186653_2...	VFG1226		twitching motility pro...	100	6e-76	
BLASTP	Virulence Factor	VFDB	Pseudomonas aeru...	fig1983918.3.peg.4647	PA13_2...	VBIPseAer186653_4...	VFG2071		Uncharacterized prot...	100	6e-119	
BLASTP	Human Homolog	Human	Pseudomonas aeru...	fig1983918.3.peg.2072	PA13_1...	VBIPseAer186653_2...	NP_071...		Methylcrotonyl-CoA c...	65	0.0	
BLASTP	Virulence Factor	VFDB	Pseudomonas aeru...	fig1983918.3.peg.2336	PA13_1...	VBIPseAer186653_2...	VFG0209		Type III secretion ne...	99	1e-168	
BLASTP	Virulence Factor	VFDB	Pseudomonas aeru...	fig1983918.3.peg.4642	PA13_2...	VBIPseAer186653_4...	VFG2076		CipB protein	99	0.0	
BLASTP	Virulence Factor	Victors	Pseudomonas aeru...	fig1983918.3.peg.3098	PA13_1...	VBIPseAer186653_3...	6537		Protocatechuate 3,4-...	82	5e-123	
BLASTP	Drug Target	DrugBank	Pseudomonas aeru...	fig1983918.3.peg.2097	PA13_1...	VBIPseAer186653_2...	VFG1264		flhF	Flagellar biosynthesi...	99	0.0
BLASTP	Virulence Factor	VFDB	Pseudomonas aeru...	fig1983918.3.peg.2354	PA13_1...	VBIPseAer186653_2...	VFG0192		Flagellum-specific AT...	99	0.0	

7. In the dynamic filter, click on Antibiotic Resistance. This will filter the table to show all the genes that meet that search criterion.



8. When you look at the column “Gene,” you can see that many of the genes do not have a gene name, although they do have a product, or functional description. Click once on the “Gene” column head.

Evidence	Property	Source	Genome Name	PATRIC ID	RefSeq Loc	Alt Locus Tag	Source ID	Gene
BLASTP	Antibiotic Resistance	CARD	Pseudomonas aeru...	fig1983918.3.peg.2...	PA13_1...	VBIPseAer186653_...	NC_00...	
BLASTP	Antibiotic Resistance	CARD	Pseudomonas aeru...	fig1983918.3.peg.4...	PA13_2...	VBIPseAer186653_...	NC_00...	
BLASTP	Antibiotic Resistance	CARD	Pseudomonas aeru...	fig1983918.3.peg.3...	PA13_2...	VBIPseAer186653_...	NC_00...	
BLASTP	Antibiotic Resistance	CARD	Pseudomonas aeru...	fig1983918.3.peg.5...	PA13_3...	VBIPseAer186653_...	NC_00...	
BLASTP	Antibiotic Resistance	CARD	Pseudomonas aeru...	fig1983918.3.peg.5...	PA13_2...	VBIPseAer186653_...	NC_00...	

9. This will resort the table and show you the genes that have an associated gene name.

Gene	Product	Pubmed	Identity	E-value
MexF	Multidrug efflux tra...	PubMed	95	0.0
MexF	Multidrug efflux tra...		87	0.0
aadA1	Streptomycin 3"-O-...	PubMed	100	0.0
aadA1	Streptomycin 3"-O-...		100	0.0
acrA	Multidrug efflux RN...		98	0.0
acrA	Multidrug efflux RN...		99	0
acrA	Multidrug efflux RN...		100	0.0
acrA	Multidrug efflux RN...		99	0.0
acrB	RND efflux system, ...	PubMed	81	0
acrB	Multidrug efflux RN...	PubMed	99	0.0

12. Examine the first row and the associated data and evidence for that gene.

Evidence	Property	Source	Genome Name	PATRIC ID	RefSeq Loc	Alt Locus Tag	Source ID	Gene	Product	Pubmed	Identity	E-value
BLASTP	Antibiotic Resistance	ARDB	Pseudomonas pseu...	fig 1182590.4.peg...	BNS_0...	VBIPsePse244945...	YP_001...	MexF	Multidrug efflux tra...	PubMed	95	0.0

13. Find the PATRIC ID and click on it.

[fig|1182590.4.peg...](#)

14. This takes you to the landing page for that gene.

Bacteria • Proteobacteria • Gammaproteobacteria • Pseudomonadales • Pseudomonadaceae • Pseudomonas • Pseudomonas pseudoalcaligenes CECT 5344 •
 fig|1182590.4.peg.1503 | BNS_01487 | VBIPsePse244945_1503 | MexF | Multidrug efflux transporter MexF

Overview Genome Browser Compare Region Viewer Pathways Transcriptomics Interactions Correlated Genes Literature

Add PATRIC Feature to Workspace

View NT Sequence
View AA Sequence

External Tools
The SEED Viewer
NCBI CDD Search
STRING: Protein-Protein Interactions
STITCH: Chemical-Protein Interactions

Recent PubMed Articles
No pubmed record is available.
Please try PMC or Google Scholar

Gene ID: **PATRIC ID:** fig|1182590.4.peg.1503 **RefSeq:** BNS_01487 **Alt Locus Tag:** VBIPsePse244945_1503
 Protein ID: **RefSeq:** CCH37584.1

MexF
CDS

Annotation	Locus Tag	Start	End	NT Length	AA Length	Product
PATRIC	VBIPsePse244945_1503	158866	162045	3180	1059	Multidrug efflux transporter MexF
RefSeq	BNS_01487	158866	162045	3180	1059	RND multidrug efflux transporter

Functional Properties

GO Assignments: -
 EC Assignments: -
 FIGfam Assignments: FIG00102465
 Pathway Assignments: -
 Structure: Not supported by SSGCID/CSGID
 Protein Interactions: **Host-pathogen interactions:** 0 **All interactions:** 0

15. Scroll down to the bottom of the page and you will see a description of the evidence that linked this gene to antibiotic resistance. It gives the specific source information, including the strain that the original gene was in, and also includes the PubMed link so that you can examine the evidence directly.

Special Properties									
Evidence	Property	Source	Source ID	Organism	PubMed	Subject Coverage	Query Coverage	Identity	E-value
BLASTP	Antibiotic Resistance	ARDB	YP_001187672	<i>Pseudomonas mendocina ymp</i>	9044268	100	100	95	0.0
BLASTP	Antibiotic Resistance	CARD	NC_002516.2.882884.p01	<i>Pseudomonas aeruginosa PAO1</i>		100	100	87	0.0

III. Assignment: Find other genes and explore them using PATRIC

You can do similar searches to find other genes of interest. Below is a list of the genes important in antibiotic resistance. For each gene, see if you can find it in the PAO1 genome, and if not, search more broadly in *Pseudomonas* or *Klebsiella*. Are any of the genes not found in either of those genera, but found in other genera of bacteria in PATRIC? Are there any genes that you can't find in any genome in PATRIC?

mexA
mexB
tolC
oprD
ampC
Aminooglycoside AAC
Aminoglycoside APH
Aminoglycoside ANT
tetM
tetO
tetQ
cfr
erm
qnrA
qnrB
qnrS
gyrA
gyrB
parC
parE
mecA
vanA
vanB
vanE
vanG
pmrA
pmrB
phoP

phoQ