Burkholderia cenocepacia str. J2315 Gene Database and Microarray Analysis of Tobramycin Treatment of Biofilm

Anu Varshneya, Brandon Litvak,
Kevin Wyllie, Veronica Pacheco
Loyola Marymount University
Department of Biology and Computer Science
Biological Databases, Fall ‘15
December 15, 2015

Outline

1. *Burkholderia cenocepacia* str. J2315 is an epidemic pathogen of cystic fibrosis patients.
2. GenMAPP Builder was customized to take in data about *B. cenocepacia*.
3. A comprehensive gene database was created using GenMAPP Builder, Gene Ontology, and Uniprot.
4. Transcriptomic analysis was performed using microarray data from Van Acker et al. (2013).
5. *B. cenocepacia* responds to tobramycin exposure by repressing the electron transport chain and protein synthesis, while inducing assembly of iron-sulfur clusters.

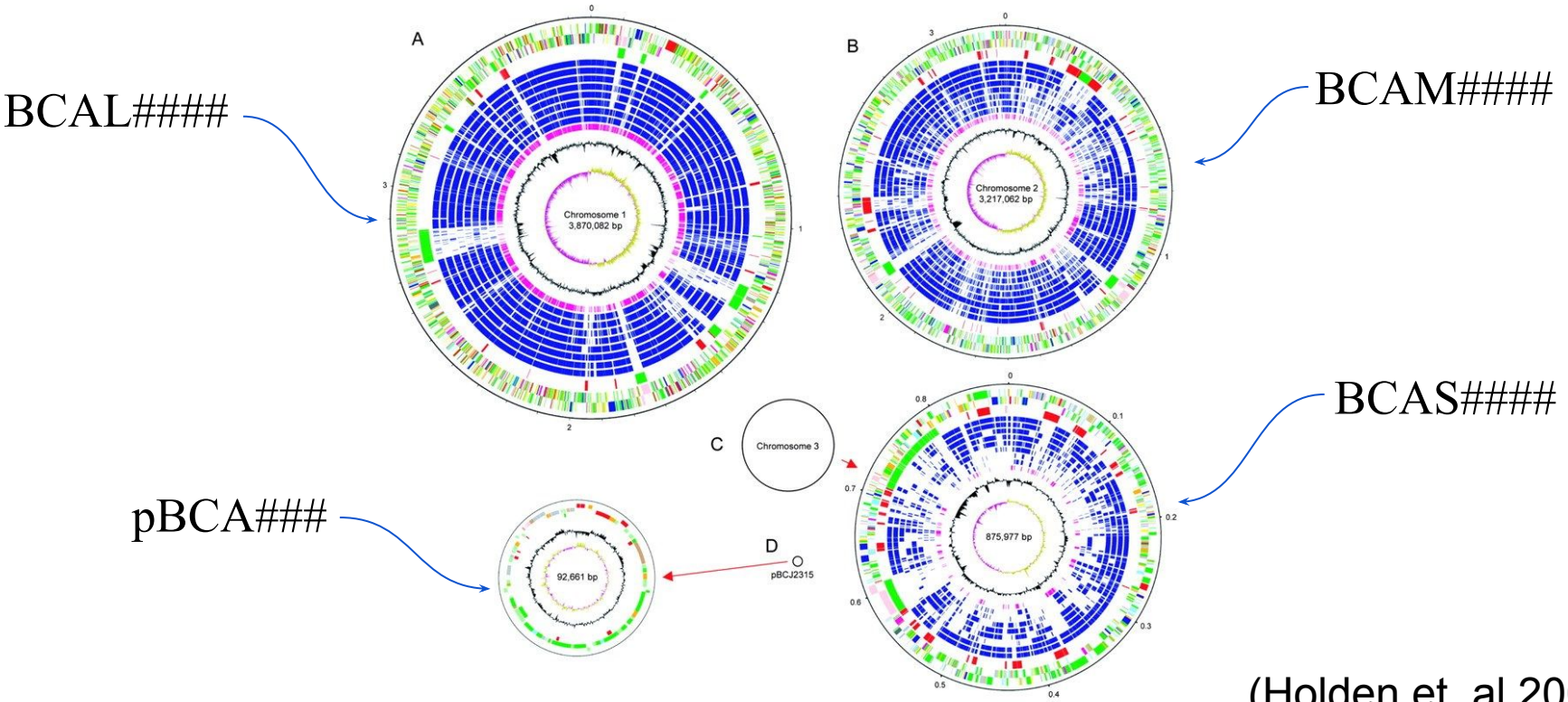
Outline

1. *Burkholderia cenocepacia* str. J2315 is an epidemic pathogen of cystic fibrosis patients.
2. GenMAPP Builder was customized to take in data about *B. cenocepacia*.
3. A comprehensive gene database was created using GenMAPP Builder, Gene Ontology, and Uniprot.
4. Transcriptomic analysis was performed using microarray data from Van Acker et al. (2013).
5. *B. cenocepacia* responds to tobramycin exposure by repressing the electron transport chain and protein synthesis, while inducing assembly of iron-sulfur clusters.

Burkholderia cenocepacia str. J2315 is an epidemic pathogen of cystic fibrosis patients

- Known to cause serious lung infections in CF patients
  - Exists as part of the B. cepacia complex (BCC)
- Belongs to the ET12 lineage of highly transmissible *B. cenocepacia* strains that leads to higher mortality rates
- Similar strains in lineage have several islands that promote pathogenic character
- J2315 associated with higher incidences of clinical deterioration and antibacterial resistance

The *B. cenocepacia* genome consists of 4 parts, each with a designated naming pattern



(Holden et. al 2009)

Outline

1. *Burkholderia cenocepacia* str. J2315 is an epidemic pathogen of cystic fibrosis patients.
2. GenMAPP Builder was customized to take in data about *B. cenocepacia*.
3. A comprehensive gene database was created using GenMAPP Builder, Gene Ontology, and Uniprot.
4. Transcriptomic analysis was performed using microarray data from Van Acker et al. (2013).
5. *B. cenocepacia* responds to tobramycin exposure by repressing the electron transport chain and protein synthesis, while inducing assembly of iron-sulfur clusters.

Several gene ID types are present in the UniProt XML for *Burkholderia cenocepacia* str. J2315

- “ORF” type gene names
  - Count of 7121
  - Form of BCAM####, BCAS####, BCAL####, pBCA###
    - Can also include letter (a-z) at the end
  - Were not originally captured by GenMAPP builder

- “ordered locus” type gene names
  - count of 337
  - Form of BceJ2315_#####
  - Were originally captured by GenMAPP builder

- All UniProt XML entries contained “ORF” gene names
  - Not all entries contained an “ordered locus” gene name

GenMAPP Builder was customized to take in data about *B. cenocepacia*

- Created a customized species profile to collect gene data from model organism database
- Added species to UniProtDatabaseProfile.java

```
6  public class BurkholderiaCenocepaciaUniProtSpeciesProfile extends UniProtSpeciesProfile {
7
8      public BurkholderiaCenocepaciaUniProtSpeciesProfile() {
9          super("Burkholderia cenocepacia",
10                 216591,
11             "This profile customizes the GenMAPP Builder export for " +
12                 "Burkholderia cenocepacia" +
13                 " data loaded from a UniProt XML file.");
14     }
15
16     @Override
17     public TableManager getSystemsTableManagerCustomizations(TableManager tableManager, DatabaseProfile dbProfile) {
18         super.getSystemsTableManagerCustomizations(tableManager, dbProfile);
19         tableManager.submit("Systems", QueryType.update, new String[][] {
20             { "SystemCode", "N" },
21             { "Species", "|" + getSpeciesName() + "|" }
22         });
23
24         tableManager.submit("Systems", QueryType.update, new String[][] {
25             { "SystemCode", "N" },
26             { "Link", "http://www.burkholderia.com/getAnnotation.do?locusID=~" }
27         });
28
29         return tableManager;
30     }
31 }
```

GenMAPP Builder was customized to take in data about *B. cenocepacia*

- Gene IDs were stored under ORF tag instead of OrderedLocusNames

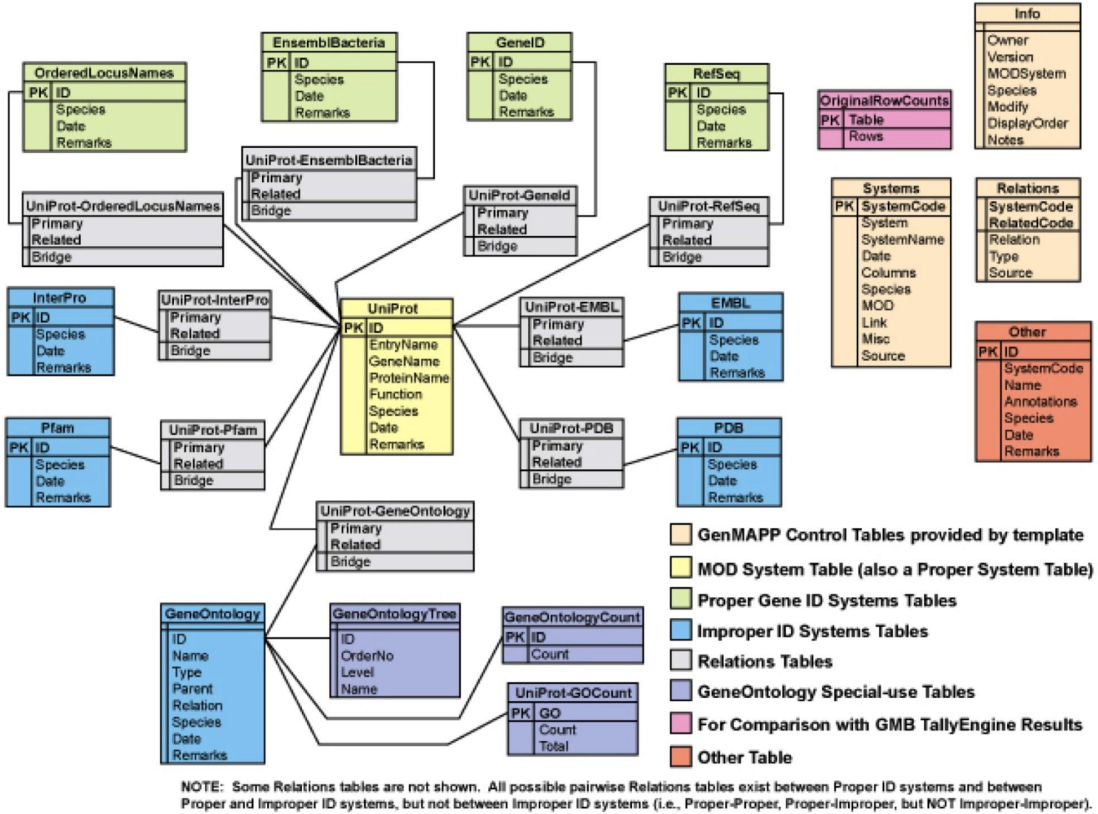
```
/**
 * Modified code from LeishmaniaMajorUniProtSpeciesProfile.java
 */
@Override
public TableManager getSystemTableManagerCustomizations(TableManager tableManager, TableManager primarySystemTableManager, Date version)
        throws SQLException, InvalidParameterException {
    List<String> comparisonList = new ArrayList<String>(1);
    comparisonList.add("ORF");

    return systemTableManagerCustomizationsHelper(tableManager, primarySystemTableManager, version, "OrderedLocusNames", comparisonList);
}
```

Outline

1. *Burkholderia cenocepacia* str. J2315 is an epidemic pathogen of cystic fibrosis patients.
2. GenMAPP Builder was customized to take in data about *B. cenocepacia*.
3. A comprehensive gene database was created using GenMAPP Builder, Gene Ontology, and Uniprot.
4. Transcriptomic analysis was performed using microarray data from Van Acker et al. (2013).
5. *B. cenocepacia* responds to tobramycin exposure by repressing the electron transport chain and protein synthesis, while inducing assembly of iron-sulfur clusters.

GenMAPP Gene Database Schema for *Burkholderia cenocepacia* str. J2315 (20151210)



*Adapted from generic species Gene Database Schema

7121 valid gene IDs were found in the exported database and in the UniProt XML file

Resource	Count of OrderedLocusNames IDs
XMLPipeDB Match, in the UniProt proteome set for J2315 (XML)	7126
TallyEngine, in the UniProt proteome set for J2315 (XML)	7121
TallyEngine, in the last created PostgreSQL database	7121
OriginalRowCounts table, in the last exported gene database for J2315	7121
Model Organism Database for *B. cenocepacia* (CDS Gene IDs)	7114

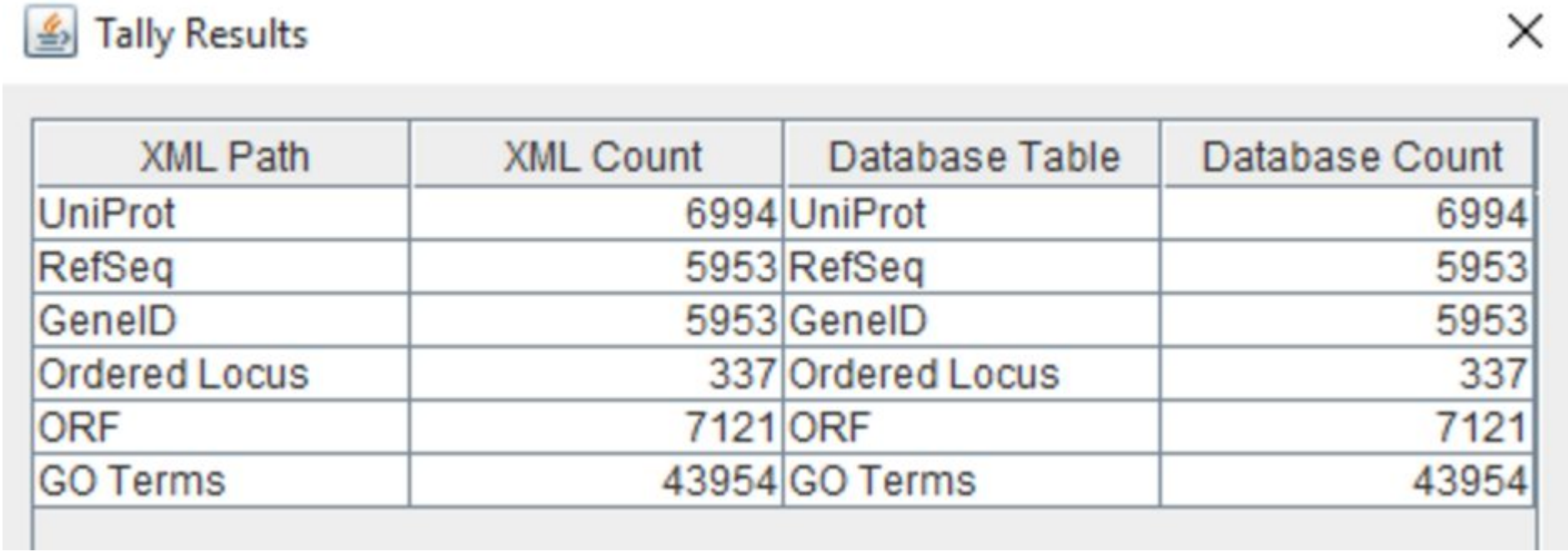
- **Utilized Match Command**:

```
java -jar xmlpipedb-match-1.1.1.jar
"p?BCA[LMS]?[0-9][0-9][0-9][Aa]?[0-9]?[A-Z,a-z]?"
< "uniprot-taxonomy%3A216591_GEN_BL12_20151119.xml"
```

- **Utilized PSQL Query**:

```
select count(*) from genenametype where type = 'ORF' and
value ~ 'p?BCA[LMS]?[0-9][0-9][0-9][Aa]?[0-9]?[A-Z,a-z]?';
```

Gene IDs from the XML file were successfully transferred into the PostgreSQL database



XML Path	XML Count	Database Table	Database Count
UniProt	6994	UniProt	6994
RefSeq	5953	RefSeq	5953
GeneID	5953	GeneID	5953
Ordered Locus	337	Ordered Locus	337
ORF	7121	ORF	7121
GO Terms	43954	GO Terms	43954

- Count of 6994 UniProt entries differs from the gene ID count of 7121
  - Was found that, in some cases, several gene IDs corresponded to one entry

263 MOD gene IDs were not included in the final gene database for *B. cenocepacia* str. J2315

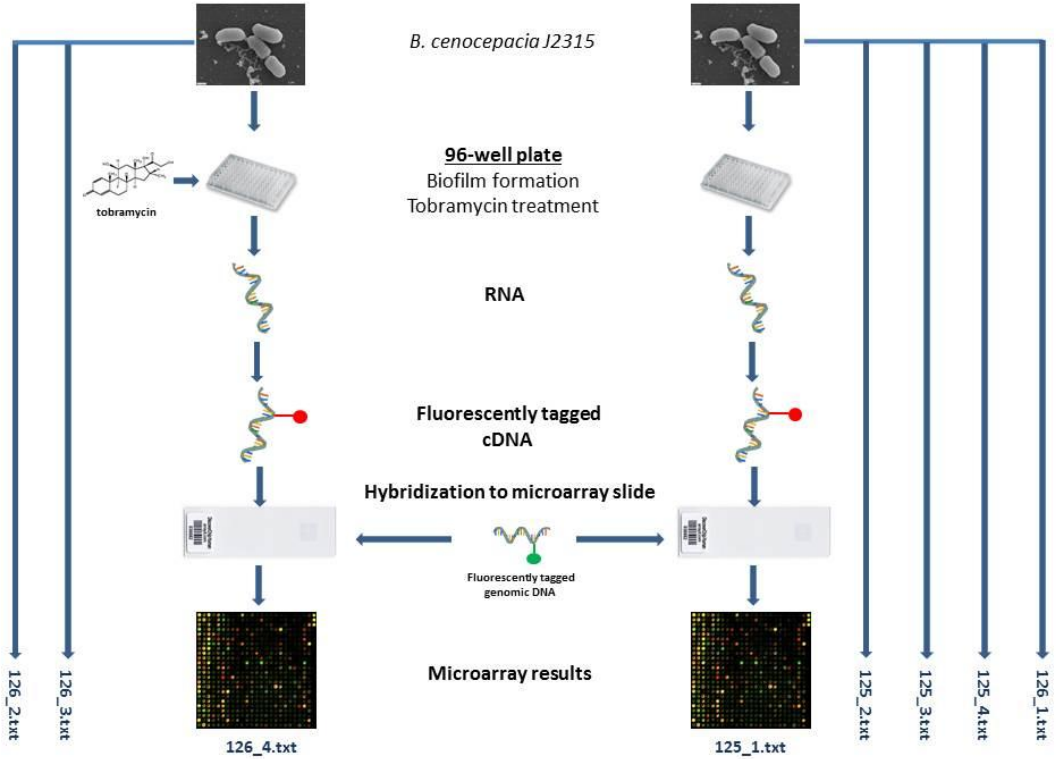
- 7121 gene IDs Present:
  - In the UniProt XML proteome set for J2315
  - In the final Postgres database
  - In the final GenMAPP gene database for J2315
- 7384 gene IDs Present:
  - In the MOD (beta.burkholderia.com)
- 263 MOD gene IDs are not present in final database:
  - Were identified as pseudo and functional RNA genes
  - Were not present in the XML source file
  - Correspond with .EX.txt file from microarray data

Outline

1. *Burkholderia cenocepacia* str. J2315 is an epidemic pathogen of cystic fibrosis patients.
2. GenMAPP Builder was customized to take in data about *B. cenocepacia*.
3. A comprehensive gene database was created using GenMAPP Builder, Gene Ontology, and Uniprot.
4. Transcriptomic analysis was performed using microarray data from Van Acker et al. (2013).
5. *B. cenocepacia* responds to tobramycin exposure by repressing the electron transport chain and protein synthesis, while inducing assembly of iron-sulfur clusters.

Van Acker *et al* performed a transcriptomic analysis on persister cells treated with tobramycin

1. *Burkholderia cenocepacia* cells grown in 96-well plate
2. Supernatant removed, treated with tobramycin
3. Persister cells harvested
4. RNA extracted, purified
5. cDNA synthesized, hybridized with genomic DNA
6. Normalization/analysis



Different significance criteria yielded varying numbers of significantly changed genes

Sanity Check	Number of Genes	Percentage out of 7251 genes (%)
P-value <0.05	4318	59.6
P-value <0.01	2350	32.4
P-value <0.001	1460	20.1
BH P-value <0.05	605	8.3
Bonferroni P-value < 0.05	179	2.5

Number of genes which exhibited an expression change considered to be statistically significant by the given criteria.

- Van Acker *et al* used a criteria of (unadjusted) P-value < 0.05
- Our criteria
  - BH P-value < 0.05
  - | Log-fold-change | > 0.25
- By our criteria, 274 (3.8%) genes were upregulated and 300 (4.1%) were downregulated
  - 574 total - 8.0%

Van Acker *et al* (2013) reported that tobramycin induced changes in cellular metabolism

- The glyoxylate shunt was induced.
- TCA and electron transport chain were repressed.
- These findings are consistent with tobramycin's proposed mechanism.
  a. Tobramycin hyper-activates NADH oxidation in the electron transport chain.
  b. This results in increased superoxide formation, damaging iron-sulfur clusters.
  c. Ferrous iron undergoes reaction which damages DNA, proteins and lipids.
- Glyoxylate shunt circumvents NADH-producing reaction in TCA.

A comparison of results shows discrepancies in significance of many genes and functions

Gene number	Annotation	Log Fold Change – GENialOMICS	Log Fold Change – Van Acker et al, Microarry	Log Fold Change – Van Acker et al, qPCR
Glyoxylate shunt				
BCAL2122	Malate synthase	0.24	1.4	−3.3
BCAL2118	Isocitrate lyase AceA	0.59	2.3	1.9
BCAM1588	Isocitrate lyase	0.78	3.1	1.6
BCAL0813	RNA polymerase factor sigma 54	−0.25	−1.5	–
BCAL1949	Glyoxylate carboligase	−0.30	−1.5	–
Tricarboxylic acid cycle				
BCAM0361	Aconitate hydratase	−0.09	−1.1	–
BCAM2701	Aconitate hydratase	−0.16	−1.3	−1.3
BCAM1833	Aconitate hydratase/methylisocitrate dehydratase	0.27	1.5	1.1
BCAL2735	Isocitrate dehydrogenase	0.20	1.3	−2.5
BCAL2736	Isocitrate dehydrogenase	0.02	1.4	1.5
BCAL1515	α-ketoglutarate dehydrogenase E1	−0.49	−2.0	–
BCAL1516	Dihydrolipoamide succinyltransferase	−0.91	−3.3	–
BCAL1517	Dihydrolipoamide dehydrogenase	−0.68	−2.5	–
BCAL2207	Putative dihydrolipoamide dehydrogenase	−0.20	−1.3	–
BCAL1215	Dihydrolipoamide dehydrogenase	−0.25	−1.4	–
BCAL0956	Succinyl-CoA synthetase beta chain	−0.50	−2.0	–
BCAL0957	Succinyl-CoA synthetase subunit alpha	−0.83	−3.3	−10.0
BCAM0967	Putative succinate dehydrogenase	−0.37	−1.7	–
BCAM0968	Putative succinate dehydrogenase	−0.66	−2.5	–
BCAM0969	Succinate dehydrogenase flavoprotein	−0.72	−2.5	
BCAM0970	Succinate dehydrogenase iron-sulfur subunit	−1.05	−5.0	−25.0
BCAL2308	Fumarate hydratase	−0.16	−1.3	1.9
BCAL2287	Putative fumarate dehydrogenase	0.02	1.0	–
BCAM0965	Malate dehydrogenase	−0.03	1.0	−2.0
BCAL2746	Putative citrate synthase	−0.14	−1.3	–
BCAM0964	Putative lyase	0.23	−1.4	–
BCAS0023	HpcH/HpaI aldolase/citrate lyase family	−0.71	−2.5	–
BCAM0372	Type II citrate synthase	−1.15	−5.0	–
Oxidative phosphorylation				
BCAL2142	Cytochrome o ubiquinol oxidase subunit III	−0.49	−2.0	–
BCAL2143	Ubiquinol oxidase polypeptide I	−0.50	−2.0	–
BCAL0750	Cytochrome c oxidase polypeptide I	−0.32	−1.7	–
BCAL0752	Cytochrome c oxidase assembly protein	−0.69	−2.5	–
BCAL0753	Hypothetical protein	−0.61	−2.5	–
BCAL0754	Putative cytochrome c oxidase subunit III	−0.47	−2.0	–
NAD(P)H production				
BCAL3276	NAD-kinase	0.22	1.4	–
BCAL0672	Isocitrate dehydrogenase kinase/phosphatase	0.21	1.3	–
BCAL3359	Glutamate dehydrogenase	1.01	4.2	–
BCAL3395	Malic enzyme	0.35	1.7	–
Response to oxidative stress				
BCAL1250	Putative glutathione S-transferase	0.35	1.6	–
BCAL3331	Putative glutathione S-transferase	0.86	3.4	–
BCAL0463	Putative thioredoxin	0.35	1.6	–
BCAL2013	AhpC/TSA family protein	0.47	1.9	–
BCAL2106	Glutathion peroxidase	0.32	1.6	–
BCAM2318	Putative ferredoxin oxidoreductase	−1.96	−10.0	−33.3
Fe-storage				
BCAM2627	Putative hemin ABC transporter protein	1.15	5.2	–
BCAM2630	Hemin importer ATP binding subunit	0.73	2.8	–
BCAM2224	Putative pyochelin receptor protein FptA	0.69	2.7	–
BCAL1790	Putative iron-transport protein	0.64	2.5	–
BCAL1347	Putative Fe uptake system extracellular binding protein	0.63	2.5	–
BCAM2228	Putative pyochelin synthetase PchF	0.01	2.1	–
BCAL1789	Putative iron-transport protein	0.48	2.0	–
BCAL1371	Putative TonB-dependent siderophore receptor	0.47	2.0	–
BCAL1702	Putative ornibactin biosynthesis protein	−0.56	−2.2	–

- Calculated log fold changes for the genes of interest in a study by Van Acker et al. (2013).
- Values shown in red were deemed significant by their respective parties.

Outline

1. *Burkholderia cenocepacia* str. J2315 is an epidemic pathogen of cystic fibrosis patients.
2. GenMAPP Builder was customized to take in data about *B. cenocepacia*.
3. A comprehensive gene database was created using GenMAPP Builder, Gene Ontology, and Uniprot.
4. Transcriptomic analysis was performed using microarray data from Van Acker et al. (2013).
5. *B. cenocepacia* responds to tobramycin exposure by repressing the electron transport chain and protein synthesis, while inducing assembly of iron-sulfur clusters.

MAPPFinder generated a list of significantly changed Gene Ontology terms

	GOID	GO Name	Number Changed	Number Measured	Number in GO	Percent Changed	Percent Present	PermuteP	AdjustedP
Increase	16226	iron-sulfur cluster assembly	7.00	8.00	8.00	87.50	100.00	0.0000	0.0090
	30288	outer membrane-bounded periplasmic space	11.00	20.00	20.00	55.00	100.00	0.0000	0.0720
	43225	anion transmembrane-transporting ATPase activity	5.00	8.00	1.00	62.50	800.00	0.0080	0.8660
	15074	DNA integration	10.00	27.00	27.00	37.04	100.00	0.0200	1.0000
	9628	response to abiotic stimulus	4.00	7.00	1.00	57.14	700.00	0.0250	1.0000
	4185	serine-type carboxypeptidase activity	4.00	8.00	8.00	50.00	100.00	0.0340	1.0000
	9086	methionine biosynthetic process	4.00	9.00	9.00	44.44	100.00	0.0590	1.0000
Decrease	43232	intracellular non-membrane-bounded organelle	25.00	68.00	57.00	36.76	119.30	0.0000	0.0000
	19843	rRNA binding	18.00	39.00	39.00	46.15	100.00	0.0000	0.0000
	6412	translation	30.00	113.00	106.00	26.55	106.60	0.0000	0.0000
	44391	ribosomal subunit	9.00	14.00	7.00	64.29	200.00	0.0000	0.0000
	16655	oxidoreductase activity, acting on NAD(P)H, quinone or similar compound as acceptor	10.00	19.00	15.00	52.63	126.67	0.0000	0.0000
	48038	quinone binding	6.00	10.00	10.00	60.00	100.00	0.0000	0.0010
	51276	chromosome organization	7.00	26.00	14.00	26.92	185.71	0.0000	0.5080
	6119	oxidative phosphorylation	4.00	7.00	7.00	57.14	100.00	0.0010	0.0540
	9082	branched-chain amino acid biosynthetic process	4.00	16.00	15.00	25.00	106.67	0.0020	1.0000

This table shows a list of gene ontology terms with corresponding pathways and/or functions considered by MAPPFinder to be significantly upregulated or downregulated in *Burkholderia cenocepacia* biofilm cells, as a response to tobramycin treatment.

Note: Error in "Percent Present" column.

A MAPP of oxidative phosphorylation shows that it was significantly downregulated

Gene Database
Bc-Std_GEN_20151204.gdb
Expression Dataset
Name: kwvp20151205
Color Set: kwvp20151205
Gene Value: kwvp20151205: biofilm_tobramycin_ratio
Legend: kwvp20151205
Increased
Decreased
No criteria met
Not found

NADH dehydrogenase

NuoA	-0.43		
NuoB	-1.04		
NuoC	-0.88		
NuoD	-1.55		
NuoE	-1		
NuoF	-0.95		
NuoG	-0.73		
NuoH	-1.07		
NuoI	-1.58		
NuoJ	-1.06		
NuoK	-1.16		
NuoL	-1.27		
NuoM	-0.85	NuoM	-1
NuoN	-0.87		

F-Type ATPase (Bacteria)

atpA	-0.69
atpB	-0.5
atpD	-0.63
atpF	-0.52
atpG	0.01
atpE	-0.56
atpH	-0.31
atpC	-0.43

Cytochrome c reductase

ISP	-0.07
Cyt b	-0.24
Cyt 1	-0.33

Cytochrome bd complex

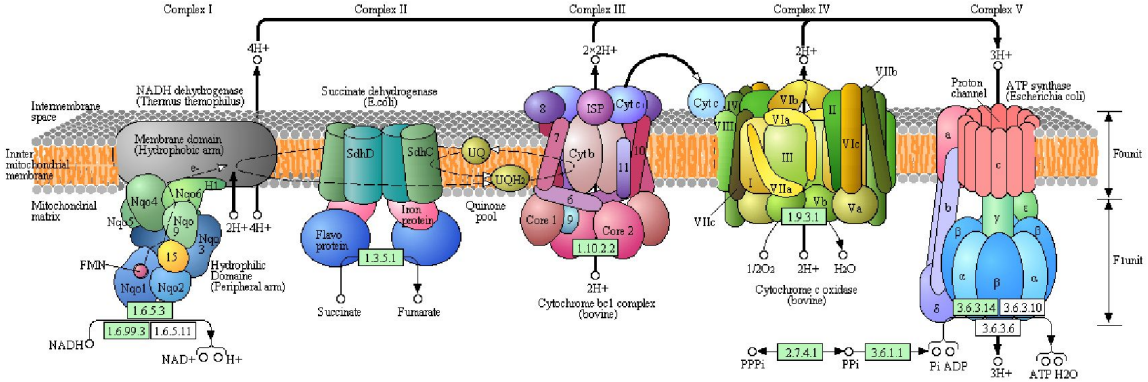
CydA	-0.06	CydA	0.36
CydB	0.01	CydB	-0.28

Succinate dehydrogenase/Fumarate reductase

sdhC	-0.37
sdhA	-0.72
sdhD	-0.66
sdhB	-1.05

Cytochrome c oxidase

COX11	-0.69		
COX15	-0.13		
CyoE	-0.27		
CyoD	-0.39		
CyoC	-0.49		
CyoB	-0.5		
CyoA	-0.29		
CoxC	-0.47	CoxC	0.16
CoxA	-0.32	CoxA	0.16
CoxB	-0.14	CoxB	0.58



Our analysis differed considerably from that of Van Acker *et al* (2013).

- With the significance criteria used by Van Acker *et al*, ~60% of *B. cenocepacia*'s genes had significant expression changes.
  - About 50/50 between upregulation and downregulation.
- With our criteria, ~8% of genes saw expression changes.
- Van Acker *et al* emphasized downregulation of TCA and electron transport chain.
- Our analysis agrees on the electron transport chain but not TCA.
- We also saw upregulation of iron-sulfur cluster synthesis, and downregulation of ribosome/protein synthesis.

Summary

- *Burkholderia cenocepacia* str. J2315 is an epidemic pathogen of cystic fibrosis patients.
- GenMAPP Builder was customized to take in data about *B. cenocepacia* by collecting gene names from ORF tags.
- A comprehensive gene database was created using GenMAPP Builder, Gene Ontology, and Uniprot indicating 7121 genes.
- Statistical analysis was performed using a more stringent significance criteria than Van Acker et al. (2013).
- *B. cenocepacia* responds to tobramycin exposure by repressing the electron transport chain and protein synthesis, while inducing assembly of iron-sulfur clusters.

Acknowledgments

Dr. Kam D. Dahlquist

Dr. John David N. Dionisio

References

Holden, M. T., Seth-Smith, H. M., Crossman, L. C., Sebaihia, M., Bentley, S. D., Cerdeño-Tárraga, A. M., ... & Parkhill, J. (2009). The genome of Burkholderia cenocepacia J2315, an epidemic pathogen of cystic fibrosis patients. Journal of bacteriology, 191(1), 261-277.

Winsor GL, Khaira B, Van Rossum T, Lo R, Whiteside MD, Brinkman FS. (2008). The Burkholderia Genome Database: facilitating flexible queries and comparative analyses. Bioinformatics 2008 Dec 1;24(23):2803-4. (PMID: 18842600)

Van Acker, H., Sass, A., Bazzini, S., De Roy, K., Udine, C., Messiaen, T., ... & Coenye, T. (2013). Biofilm-grown Burkholderia cepacia complex cells survive antibiotic treatment by avoiding production of reactive oxygen species. *PLoS One*, 8(3), e58943.

Questions?