Genome-wide association study of
Pseudomonas aeruginosa **adaptations**
to the cystic fibrosis lung

by

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects the lungs of patients with cystic fibrosis. We know that these bacteria evolve within the lung environment, and have an idea of some of the changes that occur. I performed a genome-wide association study in *P. aeruginosa* using a variety of analysis methods and several datasets of gene and SNP presence/absence. I determined that the machine learning algorithms random forest and support vector machines performed well on gene presence/absence and core SNP datasets, respectively, when compared with current methods (PLINK and treeWAS). Genes and SNPs already associated with adaptation to the CF lung environment, such as *mucA*, *gyrA*, and *mex* genes, were found with these methods. Some hypothetical and probable proteins were also recovered, and are good candidates for future research.

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1. INTRODUCTION

1.1. Cystic Fibrosis

Cystic fibrosis (CF) is a genetic disease that primarily affects lung function in afflicted individuals. It is one of the most common fatal genetic diseases [2], with greater than 1 in 3600 children in Canada born with CF [3]. Respiratory problems, such as shortness of breath, persistent coughing, and lung infections, are the most common symptoms. Other symptoms of cystic fibrosis include problems with bowel movements and digestion of nutrients [4].

CF is usually diagnosed at birth with a sweat test. In 1948 clinicians discovered that CF involves an imbalance in electrolytes, not just a defect with mucus production, such that testing the sweat can detect these imbalances and lead to a CF diagnosis. A more thorough understanding of CF and novel treatment of associated lung infections has increased the median lifespan of CF patients. In 1970, about half of patients with CF did not live to their twenties, but patients born in 2019 are expected to live until their late-thirties to mid-forties [5, 6, 7]. There is currently no cure for cystic fibrosis, only treatment of the symptoms. Gene therapies have been investigated and there is promising research with new technologies, but nothing has made it to market [8, 9].

There are over a thousand mutations in the *CFTR* (cystic fibrosis transmembrane conductance regulator) gene that cause CF, however most patients with CF have one of a select few mutations. The most common mutation is a deletion of phenylalanine at position 508, and most often affects those of European descent [4]. The *CFTR* protein regulates chlorine and sodium ion concentrations along the epithelium, and can affect the amount of liquid volume. Fluid may build in the lungs, if ion concentrations are not regulated properly, which is an ideal environment for a variety of pathogens which may cause lung infections in CF patients.

Chronic lung infections are a strong predictor of mortality in CF patients, where lung issues cause death in 80 to 95% of patients [10, 11]. Various bacteria infect the CF lung, and while some have little effect on the decline of lung function (e.g. *Stenotrophomonas maltophilia*, even when resistant to antibiotics), others have a marked effect, such as *Burkholderia cepacia* and *Pseudomonas aeruginosa* (PA) [12]. *Staphylococcus aureus* and *Haemophilus influenzae* are bacteria that usually infect CF lungs during childhood, but have not been shown to contribute to respiratory failure. They are replaced by *Pseudomonas aeruginosa* (PA) in later years, which is significantly more problematic [10].

Burkholderia cepacia is not as prevalent in CF patients, but can also cause severe lung infections and contribute to respiratory decline [13]. *B. cepacia* is especially a concern after lung transplants, while PA causes chronic infections - both contribute to the morbidity and mortality of CF patients.

1.2. *Pseudomonas aeruginosa*

PA is a ubiquitous bacterium that has been found to undergo genetic level changes after infecting the CF lung. In later stages of colonization, infections become chronic and impossible to clear [11, 12, 14, 15]. PA infections typically becomes a chronic during a patient's adolescence [6, 14]. Once a chronic infection takes hold, the goal of clinicians is no longer to clear the infection, but rather to manage it and try to keep lung function high. [12]. One theory for the difficulty in clearing bacterial infections is that the microbes get trapped in the lung fluid due to it being less fluid than that of healthy people. In addition, airways of CF patients have a reduced ability to clear invading bacteria [12]. Many antimicrobial treatments are used to treat these infections - preference is to use aerosol therapies as these can directly target the lung sputum while avoiding systemic adverse effects. However in more severe infections intravenous antibiotics are needed [12]. Intermittent infections can be effectively treated by antibiotics and this delays the onset of chronic infection. When reinfection occurs, 25% of the time PA will have the same genotype, suggesting that the initial infection was not completely eliminated [6]. If PA has a different genotype, it suggests new colonization from the environment, or that another undetected genotype within the patient has re-infected the host [6].

PA has been widely studied in the context of CF. There are longitudinal studies that will follow one or a few patients and their infections over time [16, 17, 18, 19], studies of epidemic strains [20, 21] and samples from CF clinics [11, 22, 23]. This has helped characterize the evolution and colonization of PA in a CF lung environment. Acquisition of PA infections in CF patients is usually from the environment, not from patient to patient transfer [7, 23].

There are significant trends in PA in the adaptation to their environment in chronic infections. Strains often become mucoid during the early stages of a chronic infection, but may lose this mucoidy in late infections [7, 17, 24]. They will form biofilms, develop resistance to antibiotics, adapt metabolic pathways, change in virulence, and have an increased mutation rate [6]. This evidence for convergent evolution among chronic PA strains emphasizes a need to understand

the parameters driving evolution and the adaptations that arise to better control and treat these infections.

PA may become mucoid and form biofilms after chronic infection, making the infections harder to clear. Mucoidy is caused by an overproduction of alginate, which creates a glycocalyx around the bacterium [6]. *muc* genes regulate alginate production genes (*alg*, most importantly *algD* and *algU*). Loss of function mutations in *muc* genes can lead to the formation of mucoid mutants [24, 7]. Many of these mutations are found within the *mucA* gene. It is not uncommon for PA to revert back to a non-mucoid phenotype after chronic infection, either via a back-mutation or a secondary mutation in another gene [6, 7, 24]. This is due to a change in selective pressures in the environment and the fact that producing more alginate can be costly for the organism.

The lack of regulation of *algU* can lead to other adaptations, in addition to mucoidy. These changes include increases in heat shock, oxidative-stress and osmotic-stress responses, while inhibiting the type 3 secretion system and the Rhl quorum sensing signal [6, 7]. A higher production of alginate protects the bacteria from environmental stress and harsh lung conditions during a host inflammation response [6, 7].

PA forms subpopulations within a CF lung with different phenotypes. Part of this is due to a diverse respiratory environment, where, for example, the nasal passages have different nutrients compared to the lung alveoli [6]. Some subpopulations will become specialists in their niche, while others will remain generalists. Different populations may lose or gain functions to help the population of PA survive - some may be more mobile, some may be better suited to an anaerobic environment, while others may be more virulent.

Some subpopulations form small colony variants, made up of adherent bacterial cells, possibly due to the upregulation of pellicle and extracellular matrix genes (*pel* and *psl*) [7, 24]. Small colony variants are able to persist in the lung environment longer and have greater resistance to antibiotics [25]. In addition, isolates with mutations in *pel* and *psl* genes are better suited to form biofilms, having developed an aggregate population already [7, 24].

Many mucoid strains of PA have reduced motility, since biofilms reduce the need for movement. Biofilms are communities of bacteria where there is an extracellular matrix of polysaccharides, proteins and DNA. Flagella are often repressed, but strains can re-gain their flagella with the reversion of mucoidy [7, 24]. The microbes in a biofilm are much more resistant to antibiotics than

free-living microbes. It is more challenging for antibiotics to move through the extracellular matrix in a biofilm, thus they cannot reach their target of action. Biofilms also have novel methods to decrease their sensitivity to antibiotics. *ndvB* is involved in the production of periplasmic glucans, which are thought to bind to antimicrobials and prevent them from binding to their target site [26]. Two-component regulatory systems involved in biofilm formation may also play a role in antibiotic resistance. *tctD* and *tcdE*, when present, reduce the expression of porin OmpD, which in turn reduces the isolate's susceptibility to antibiotics [27, 28].

Biofilms are formed when there is an increase in alginate and the bacteria become more mucoid. Biofilm formation prompts a strong immune response from a host and often defines the beginning of a chronic PA infection in CF patients [6, 7]. Extracellular components can also be shared by the bacteria forming the biofilm, such as quorum sensing molecules [7], meaning biofilms can house heterogeneous PA communities that work together to benefit the population. There can be differences in nutrient usage, energy expenditure, and antibiotic resistance profile in different locations within the biofilm [7].

PA is able to chronically infect the CF lung by adhering to the lung epithelium, losing its motility, and evading the immune system. These functions are believed to have related methods-of-action: in the overproduction of alginate, the flagellum formation is suppressed to re-direct energy expenditure towards alginate production. This allows the bacterium to embed itself in the epithelial lung cells, and allow it to go unrecognized by the immune system [7, 24]. Other adaptations can also increase the success of PA colonization, such as the loss of pili (mutations or losses of *pilB*, *pilQ*, or *rpoN*) which aids in the adherence of the bacteria to the lung.

Acute and chronic PA infections are treated with a variety of antibiotics. The bacteria become resistant over time, especially in the case of chronic infections. Longitudinal studies have repeatedly observed the evolution of resistance by sampling isolates from a single patient over the course of several years [16, 17, 19]. There are a variety of mechanisms that confer antimicrobial resistance (AMR), and these mutations within CF PA isolates are related to the treatment an individual has received over the course of their infection. AMR-conferring mutations are generally *de novo* since guidelines for people living with CF has limited the transfer of infections between individuals [6].

The evolution of PA resistance to aminoglycosides, beta-lactams, ciprofloxacin, colistin, tobramycin, and others (including multi-drug resistance) has been observed and studied in previous

years [7]. Mutations that confer resistance co-evolve in various lineages of PA.

Many of the mutations that confer resistance are found in efflux pumps (*mex* genes) or porins (*omp* or *phoE* genes). Changes to the functionality of these proteins can provide multi-drug resistance since the bacterium will have decreased uptake and increased expulsion of the drug. Beta-lactams, carbapenems and fluoroquinolones are some of the drug classes affected by these changes [29, 30]. Mutations in *gyrA*, *gyrB*, *parC* or *parE*, which encode for drug target enzymes DNA gyrase and topoisomerase IV, can cause resistance to fluoroquinolones [31]. Common resistance mutations include S83L, S83R and D87Y, which alter the binding site of quinolones in gyrase A or topoisomerase IV [32, 33, 34]. Similarly, resistance can be caused by deletions and SNPs in other proteins, such as transcription (e.g. S531L in *rpoB*) and translation factors (e.g. T12R in *fnt* or P413L in *fusA*), which confer resistance to specific antibiotics [34]. Overexpression of *ampC* results in resistance to beta-lactams [35, 36].

Many CF-related adaptations in PA are associated with metabolic pathways. Many chronic PA strains become unable to produce all required amino acids [24]. Changes to the PQS (*Pseudomonas* quinone signal), a quorum sensing molecule, are also common. Both mutations and fluctuations in nutrition can alter the PQS, which in turn causes changes to gene expression and regulation in the bacteria [24]. PA in chronic affections appear to prefer amino acids over glucose as a source of carbon [24]. Increased expression of *oprF*, an outer membrane protein, can increase antibody production of the host and allow growth in a microaerobic or anaerobic environment [6, 7, 24].

In Gram-negative bacteria, such as PA, one of the components in the outer membrane is lipopolysaccharide (LPS). The LPS has three parts: an interior lipid A section that is embedded in the outer membrane, a central polysaccharide, and an O sidechain (antigen O). The O side chain is recognized by a host immune system and can change rapidly in bacteria to evade capture. The lipid A section can be toxic and cause immune responses from the host. In PA, changes to certain genes can alter the O lateral chain part of the LPS, which helps it remain undetected by the immune system [24].

Virulence often decreases in chronic infections of PA. There is lower production of pyoverdine, pyocins and changes to the type II and type III secretion systems (T2SS and T3SS respectively). This results in less tissue necrosis by PA in a chronic infection than during an acute infection. Loss-of-function of *lasR* accounts for some of the secretion system changes. LasR is a quorum

sensing regulator, which activates other virulence factors [6, 24]. Exotoxin genes are also known to be down-regulated or lose function during chronic infection (*exoS*, *exoT*, *exoY*) due to changes in the T3SS which decreases the toxicity of the bacterial cells.

Mutators are often observed in CF-related infections and biofilms. They have fitness advantages in chronic infections due to their ability to rapidly adapt to new environments. Mutator strains can have a 1000-fold increase in the rate of mutation [7]. Changes in the rate of mutation are due to mutations or recombinations in DNA mismatch repair and error detection genes [11]. Environmental factors can elicit this change by prompting microevolutionary changes, such as the presence of damaging reactive oxygen species from an immune response or the presence of antibiotics. *mut* genes (especially *mutL* and *mutS*), as well as *uvrD* are often responsible for a mutator phenotype [7]. Although adaptation of PA to a CF lung environment has been studied, we do not yet know all of the mechanisms involved.

1.2.1 Genome structure of *P. aeruginosa*

PA has a genome of 5.5 to 7Mb, arranged in a single circular chromosome. The genome contains around 6000 genes, but this varies from strain to strain [37]. A core genome size of around 4800-5200 genes is consistent in the literature [37, 38, 39, 40, 41]. Bacterial strains of the same species do not share 100% of genes in common, unlike eukaryotes. Thus, the genome is split into two categories, 'core' and 'accessory'. The core genome is that shared by all strains within the species, however this definition is flexible. Usually studies will use a threshold of less than 100% to account for phylogenetic outliers as well as possible errors with annotation or assembly. The true core genome of bacterial species is widely debated [37, 42, 43]. The accessory genome is comprised of the genes that are shared by some, but not all, strains within a species. The accessory together with the core genome is called the 'pan-genome'.

Several studies have defined the PA core genome as 4.8-5.9 Mbp in length [41, 40, 39, 37]. The pangenome in each of these studies increased as more isolates were sampled [37, 39]. The core genome is comprised of a large number of known metabolic genes (about 80% of the metabolic genes are in the core genome), cellular signalling and information storage/processing [37]. A number of core genes and most of the accessory genes have an unknown function.

A few other studies have suggested that the core genome harbours fewer than 4800 genes. A

study using 181 genomes determined a core genome of 2503 genes in 100% of their strains [43]. A more recent study found an even smaller core genome with 665 genes from 1311 strains [42]. These 100% cutoffs are too stringent, potentially introducing biases with the samples and sequencing methods used. If a strain is rare and does not have long-term viability but was sampled, genes that are necessary for survival might be excluded. In addition, the dataset would be susceptible to poor assemblies and outliers [44, 45].

PA strains can generally be classified into three groups, based on their reference - PAO1-like, PA14-like, or PA7-like. PAO1 and PA14 are common reference strains that are quite similar [35]. PA7 has been found to be a distant phylogenetic outlier when compared with other PA strains, however it remains in a clade with PA when compared with other species of bacteria [38]. In this strain, robust sections of the genome have been deleted, and there are unique genomic islands.

1.3. Genome-wide association studies

Genome-wide association studies (GWAS) have identified causal genomic variants in many human diseases over the past decade and a half [46, 47]. GWAS find correlations between genes or genetic mutations, and diseases. They look for higher incidences of genetic variants in a case (disease afflicted) group compared to a control (non-afflicted) group. Generally these variants are single-nucleotide polymorphisms (SNPs) or copy-number variants (CNVs). GWAS have been successful in identifying genes that contribute to and/or are responsible for type I and type II diabetes, Alzheimer's disease, Parkinson's disease, heart disease, prostate and breast cancer, to name a few [46]. There have been several challenges with GWAS in human studies due to the nature of genomic data. Linkage disequilibrium, population structure and complex traits are the most common concerns affecting GWAS, and have been addressed in various ways over the years.

In human germ cells, chromosomes undergo recombination where homologous chromosomes cross-over and exchange genetic material. This contributes to genetic variation in the population. If two alleles or loci assort independently in a population, they are said to be in linkage equilibrium. Such alleles appear no more often together during sampling than they would when sampled randomly. If two alleles are in linkage disequilibrium (LD), they are found more often together during sampling than expected. This may be because they are located close to each other on a chromosome. During recombination, chromosomes cross-over and exchange genetic material - if

two loci are far apart, they will often end up in linkage equilibrium in the population. If they are close together, it is more unlikely that cross-overs will occur between them, and they will remain linked for a longer period of time.

LD can be an issue in human GWAS when several alleles/mutations both appear to be significantly associated with a phenotype, but are in high LD with one another. This generally means that not all of them cause the phenotype, but rather that some of the mutations just ‘piggyback’ during recombination events due to their close proximity on the chromosome. It is important to distinguish these spurious results from the alleles/mutations associated with the phenotype. As a resolution to this issue, the software package PLINK [48, 49] calculates LD between SNPs within a given nucleotide window and gives the option to exclude SNPs that are in LD with one another.

Population structure can be a significant challenge when detecting associations in GWAS. If multiple populations are sampled (for example, a dataset has samples from both European and African genomes), important SNPs may be lost if they only contribute to the phenotype in one population, not both. Conversely, if there are more cases sampled from one population, then variants distinguishing subpopulations may be spuriously associated with the disease. There are many methods that can currently account for population structure and remove these false negatives and positives [50, 51]. Some methods have used principal component analysis (PCA) to account for variation in subpopulations (SMARTPCA [52] and EIGENSTRAT [53]), while others use Bayesian inferences (FastSTRUCTURE [54]) or maximum likelihood models (ADMIXTURE [55]) to infer population structure.

Many human diseases are complex traits, caused by multiple mutations and varying degrees of affliction. When testing for genetic associations to a phenotype, it can be challenging to account for epistasis and interactions between genes [56]. If phenotypes are caused by two-gene interactions, it may be possible to calculate all possible iterations, given time. However with an increased number of interactions and exponential growth in the amount of calculations needed, computational time and resources can be limiting.

Disease-causing SNPs can be rare in a population, resulting in low power to detect associations. In some cases, this can be avoided by using larger datasets. General practice, however, removes variants that are in less than 1% of the population studied. This decreases computation time and power, as well as removes some false positives. In doing so, true positives may also be inadvertently

excluded [56, 57, 58].

1.3.1 Bacterial GWAS

In recent years, GWAS have expanded to other organisms, such as plants (e.g. [59]), fungi (e.g. [60]), and bacteria (e.g. [61, 62]). Challenges associated with human GWAS analyses are still prevalent microbial GWAS, but differences between genome and microbial structure can present some unique issues.

Bacteria are predominantly asexual organisms, and usually have haploid circular chromosomes. This differs from humans, who undergo sexual reproduction and have linear, diploid chromosomes. This means there can be long-range LD in bacterial genomes, unlike in human genomes where LD is maintained over short distances. Population structure still plays an important role in bacteria, but can be difficult to determine and verify, unlike in human studies where we can get information about ancestry from a patient to compare against results from population stratification methods. In addition to LD and population structure, genetic drift and natural selection can play a more important role in these large populations with shorter generation times. Genomes are more varied between different bacterial isolates within a species - genes are not always in the same order, and not all genes are shared by all specimens. This can make alignment and genome comparison more challenging.

If bacteria are purely clonal (there is no recombination), the whole genome is in LD. Recombination breaks up this LD, and can vary widely between different bacterial species [61]. Although LD can be accounted for in programs like PLINK [48], if an organism has a highly clonal genome it can still be problematic to find which variants are causal, as found with *Mycobacterium tuberculosis* [61].

Natural selection can encourage similar populations via positive selection. This can create subpopulations that are genetically alike, but it can be difficult to tell if genome similarity is because of shared ancestry or because of natural selection [61, 63]. Studies have used linear mixed models [63, 64], PCA [65, 66], phylogeny [67, 68], and various programs (e.g. PLINK) to account for population structure.

Another challenge in population structure with bacteria is the possibility of within-host evolution. Some species of bacteria are known to form sub-populations within their host, with some isolates become specialists for their environment, and others generalists, such is the case with PA. A given

sample may not reflect the diversity within the environment or contain the necessary genetic elements that contribute to an observed phenotype [44]. This is a problem in current research of the microbiome as well, with limited solutions [69].

As mentioned previously, genomes can vary widely between strains of a single bacterial species. This, coupled with higher rates of recombination, can make whole-genome alignment challenging. There are programs that can handle aligning sequences that appear in different parts of a genome (e.g. MAUVE [70]), but performing such alignments is computationally intense with an increasing number of sequences. Bacterial GWAS systems have handled the problem primarily in three ways - aligning orthologous genes against each other and using gene presence/absence information in the core genome [65, 71]; using SNP information in just the core genome [61, 72]; or using *k-mers* [73], where sequences are split into smaller 'words' (10-30bp in length), and these shorter sequences are grouped with other similar *k-mers* and counted. Each of these methods has strengths and weaknesses - *k-mers* include the most amount of information, but it can be difficult to translate back into gene function; the other two methods may omit information that could include causal variants.

There have been an increasing number of bacterial GWAS published in the last five years, as more whole genomes are being sequenced (Figure 1, Table 1). Each study has addressed challenges, such as population structure or high LD, in different ways. A benefit to bacterial GWAS over human studies is that the results can be explored and verified through genomic manipulation and growth in the laboratory, while in human GWAS it can be very difficult to confirm correlations between SNPs and a phenotype.

Table 1: Published bacterial GWAS, by year. Number of variants is number of genes, SNPs or k-mers analyzed.

<i>Study</i>	<i>Year</i>	<i>Taxa</i>	<i>Number of genomes</i>	<i>Number of variants</i>	<i>Phenotype</i>	<i>Methods</i>
van Hemert <i>et al.</i> [74]	2010	<i>L. plantarum</i>	42	?	Host immune response	Random forest (in R)
Sheppard <i>et al.</i> [73]	2013	<i>C. jejuni</i>	192	?	Host specificity	Bespoke
Stoesser <i>et al.</i> [75]	2013	<i>E. coli</i> ; <i>K. pneumoniae</i>	74; 69	100; 100	AMR	<i>bugwas</i> (in R)
Farhat <i>et al.</i> [72]	2013	<i>M. tuberculosis</i>	123	25,000	AMR	PhyC
Salipante <i>et al.</i> [71]	2014	<i>E. coli</i>	312	15,000	AMR	Logistic regression (in R)
Alam <i>et al.</i> [76]	2014	<i>S. aureus</i>	75	55,000	AMR	ROADTRIPS
Gordon <i>et al.</i> [77]	2014	<i>S. aureus</i>	501	?	AMR	<i>bugwas</i> (in R)
Laabei <i>et al.</i> [78]	2014	<i>S. aureus</i>	90	3000	Virulence	PLINK
Chewapreecha <i>et al.</i> [79]	2014	<i>S. pneumoniae</i>	3701	400,000	AMR	PLINK
Chaston <i>et al.</i> [80]	2014	various	41	12,000	Host development time and triglyceride content	Linear mixed model (in R)
Chen & Shapiro [61]	2015	<i>M. tuberculosis</i>	123	3000	AMR	PLINK
Walker <i>et al.</i> [81]	2015	<i>M. tuberculosis</i>	3651	5000	AMR	<i>bugwas</i> (in R)
Miotto <i>et al.</i> [64]	2015	<i>P. falciparum</i>	1063	18,000	AMR	FaST-LMM
Desjardins <i>et al.</i> [82]	2016	<i>M. tuberculosis</i>	498	11,500	AMR	PhyC
Cohen <i>et al.</i> [83]	2016	<i>M. tuberculosis</i>	91	17,500	AMR	Linear mixed model
Lees <i>et al.</i> [84]	2016	<i>S. pyogenes</i>	3069	68,000,000	AMR; virulence	SEER
Suzuki <i>et al.</i> [85]	2016	<i>A. baumannii</i>	122	2,125,000	AMR	<i>bugwas</i> (in R)
Bao <i>et al.</i> [86]	2016	<i>S. pyogenes</i>	44	71,500	Host adaptation	Chi-squared test
Porter <i>et al.</i> [87]	2017	<i>Mesorhizobium</i>	53	225,000	Nickel adaptation	non-parametric Wilcoxon rank sum test
Kremer <i>et al.</i> [88]	2017	<i>L. monocytogenes</i>	96	169,000	Meningitis	GEMMA
Mobegi <i>et al.</i> [89]	2017	<i>S. pneumoniae</i>	1680	124,000	AMR	PLINK, random forest (in R)
Skwark <i>et al.</i> [65]	2017	<i>S. pneumoniae</i>	3156	81,000	Selection	genomeDCA
Lees <i>et al.</i> [90]	2017	<i>S. pneumoniae</i>	4382	>5,250,000	Carriage duration	FaST-LMM; SEER

<i>Study</i>	<i>Year</i>	<i>Taxa</i>	<i>Number of genomes</i>	<i>Number of variants</i>	<i>Phenotype</i>	<i>Methods</i>
Swings <i>et al.</i> [91]	2017	<i>E. coli</i>	16	4500	Ethanol tolerance	IAMBEE
He <i>et al.</i> [92]	2017	<i>E. coli</i> ; <i>S. aureus</i>	45; 45	169,000; 84,000	Growth	Q-ROADTRIPS
Bethernet <i>et al.</i> [93]	2018	<i>H. pylori</i>	565	27,500	Gastric cancer risk	<i>bugwas</i> (in R)
Coll <i>et al.</i> [94]	2018	<i>M. tuberculosis</i>	6465	113,500	AMR	PhyC
Collins & Didelot [67]	2018	<i>N. meningitidis</i>	171	170,000	AMR	treeWAS
Wheeler <i>et al.</i> [95]	2018	<i>S. enterica</i>	13	6500	Host adaptation	Random forest (in R)
Puranen <i>et al.</i> [66]	2018	<i>S. pneumoniae</i>	3042	94,000	Selection	SuperDCA
Kavvas <i>et al.</i> [96]	2018	<i>M. tuberculosis</i>	1595	?	AMR	Support vector machine (in R)
Diaz Caballero <i>et al.</i> [97]	2018	<i>B. multivorans</i>	111	1800	AMR	Fisher's exact test
Epstein <i>et al.</i> [98]	2018	<i>E. meliloti</i>	153	124,000	20 different phenotypes	GEMMA
Ruesen <i>et al.</i> [99]	2018	<i>M. tuberculosis</i>	322	6500	Meningitis	independent algorithm
Gori <i>et al.</i> [100]	2019	<i>S. agalactiae</i>	1988	?	clonal characterization	Scoary
Buron-Moles <i>et al.</i> [101]	2019	various	56	6000	lactic acid metabolism	non-parametric Wilcoxon rank sum test

1.4. Machine Learning

"Machine learning" refers to a family of artificial intelligence methods that create a model to categorize or classify data points within a dataset. This model can, in some cases, be broken down to determine what features or variables are important for the data classification. This technology is not new, but it has taken a few decades to become more mainstream. It is currently used in many fields, from predicting fraudulent credit card transactions, to recommending advertisements to users, to predicting protein-protein interactions [102].

There are many different algorithms under the umbrella term *machine learning*, including models such as random forest, support vector machines, neural networks, and k-nearest neighbour. Each method has strengths and weaknesses based on the amount and type of data. For GWAS, we want a classifier that can predict a phenotype and tell us what information was needed to create the model (what features or variants - in this case genetic variants - are important for predicting a phenotype). With supervised machine learning methods, the data are split into training and testing sets, where the algorithm is created knowing all the information about the training dataset, and then that algorithm is tested on the testing data where the phenotype is hidden. An accuracy score is produced based on how often the model was correct for the testing set when compared with the true phenotype. Machine learning methods are particularly valuable for large datasets with a large number of features, where there may be complex solutions that cannot be determined through other methods. Many of these algorithms are very fast, even with thousands of features.

1.4.1 Random Forest

Random forest (RF) is a machine learning algorithm that creates a number of *decision trees* and outputs the mean of the individual trees [103]. A decision tree splits the dataset based on a feature (for example, in a GWAS - presence/absence of a gene) that will maintain the most accuracy in the model. Features that are more basal contribute more to the accuracy of the final prediction. Decision trees take into account multiple features at a time to predict the final phenotype (e.g. presence of Gene A + absence of Gene B + absence of Gene C = 90% confidence that the individual would be resistant to antibiotics, see Figure 2). Random forests take a group of decision trees, and output the importance of each feature in the model. The importance is either the mean decrease in accuracy over all the decision trees, or the mean decrease in node impurity (measured using the Gini index).

The Gini index is a calculation of how homogenous the subsets of data are after a given split.

1.4.2 Support Vector Machine

Support vector machine (SVM) models are another supervised learning method where the samples are plotted in a multi-dimensional space using all the features available [104]. A hyperplane in that multi-dimensional space is drawn to split the points based on their phenotype, ideally with all cases on one side of the hyperplane and controls on the other. An equation for the hyperplane is calculated, with a coefficient for each feature. The hyperplane coefficient is used to determine which features were most important in splitting the data - the further away from zero the coefficient, the more a variant contributed to splitting the data (Figure 3).

1.4.3 Machine Learning and GWAS

A large problem with statistical testing is in large p , small n problems, where the number of variables tested, p , is much larger than the number of samples, n . Many machine learning methods can tackle these problems well, by ignoring variables that do not contribute to the model, ie. feature selection. This is perfect for GWAS, where the number of samples is often much smaller than the number of variants investigated.

The adoption of machine learning methods by the GWAS community has been slow, but has gained traction within the last few years. A decade ago, various machine learning models were tried on previously tested datasets, and found similar or improved results with various methods [105]. The main problem encountered with RF and SVM models was the lack of conversion to p values from importance scores or hyperplane coefficients. These models were largely able to find causal variants, even among many irrelevant SNPs. A more recent human GWAS applied an SVM to a previously explored dataset to eliminate variables that are unnecessary to the model, then calculated p values for the remaining SNPs. They found all of the initially discovered SNPs, plus a few that had not been identified in the original analysis [106].

There have been a few bacterial GWAS that have used machine learning methods in the past two years. A study of *S. pneumoniae* used PLINK [48] to select for genes and SNPs that may confer antibiotic resistance, then used an RF to see if there were any important interactions between them [89]. A different study of *M. tuberculosis* used an SVM to find genes that contribute to antibiotic

Figure 2: Example of decision tree, where the dataset is split on gene presence (1) or absence (0) to make homogenous groups of antimicrobial resistant (AMR) and non-resistant (no AMR) strains.

Figure 3: Example of support vector machine model with three variables: x , y and z . Strains with phenotype blue and orange cannot be split by x and y (Panel 1), however they can be clearly split on axis z (Panel 2).

resistance with success [96]. Both studies had around 1600 samples.

Two other recent studies used random forest to look for gene associations in bacteria, however their sample sizes were much smaller. A study of *S. enterica* had only 13 samples, but was able to identify features putatively contributing to host adaptation. A study of Influenza A and *P. aeruginosa* had mixed results [107]. They were able to find associations between host adaptation and genes using repeated random forest, with only 10 strains of influenza. However, with only 26 *P. aeruginosa* genomes, they were unable to converge on an consistent model to find genes associated with AMR. This emphasizes the need for large datasets where possible (hundreds or thousands of samples), even when machine learning algorithms can handle large n small p problems.

1.5. Motivations

To find novel genes associated with CF adaptation, I have carried out a genome-wide association study with *P. aeruginosa*. The goal is to identify potential genes or SNPs associated with adaptation of the bacterium to a CF lung environment. Ultimately, such information may lead to new avenues to treat PA infections. With improved capabilities to treat chronic lung infections, the expected lifespan of a person living with CF can be extended.

I used a variety of approaches to explore which methods perform the best for this, and possibly future, bacterial GWAS. I used a number of different datasets to examine how different datasets and methods affects the results, and identify the most promising gene and SNP candidates in PA for further laboratory research.

2. METHODS

2.1. Data collection and dataset curation

2225 annotated genomes were obtained from the online *Pseudomonas* database [1], accessed October 28th, 2017. Strains with missing source information were removed, leaving 1597 genomes. The remaining strains were classified as isolated from CF patients ("CF" hereafter), or as isolated from other sources ("non-CF" hereafter). The non-CF category thus encompasses isolates from environmental sources and from acute, non CF-related, infections.

Genes in these 1597 genomes had been annotated by various studies using one of 28 reference

strains (Table 2). I cross-referenced these annotations to identify orthologs between strains, mapping to a reference strain, PAO1. Genes that had no ortholog from annotation were BLASTed against PAO1 genes, using an e-value cutoff of 0.001. If the PAO1 reference hit length was greater than 50% of the of the query gene length, the match was noted. The longest match with the lowest e-value was identified as the ortholog for each query gene.

Table 2: Reference strains used for annotations in various studies. Known orthologs with PAO1 were used to identify genes in the 1597 genomes.

<i>Strain</i>
<i>P. aeruginosa</i> 19BR
<i>P. aeruginosa</i> 213BR
<i>P. aeruginosa</i> B136-33
<i>P. aeruginosa</i> Carb01 63
<i>P. aeruginosa</i> DK2
<i>P. aeruginosa</i> DSM 50071 - Assembly GCF_001045685.1
<i>P. aeruginosa</i> F22031
<i>P. aeruginosa</i> F9676
<i>P. aeruginosa</i> FRD1 - Assembly GCF_000829885.1
<i>P. aeruginosa</i> LES400
<i>P. aeruginosa</i> LES431
<i>P. aeruginosa</i> LESB58
<i>P. aeruginosa</i> LESlike4
<i>P. aeruginosa</i> LESlike5
<i>P. aeruginosa</i> LESlike7
<i>P. aeruginosa</i> M18
<i>P. aeruginosa</i> MTB-1
<i>P. aeruginosa</i> NCGM1900
<i>P. aeruginosa</i> NCGM1984
<i>P. aeruginosa</i> NCGM2.S1
<i>P. aeruginosa</i> PA1R
<i>P. aeruginosa</i> PA7
<i>P. aeruginosa</i> PACS2
<i>P. aeruginosa</i> RP73
<i>P. aeruginosa</i> SCV20265
<i>P. aeruginosa</i> UCBPP-PA14
<i>P. aeruginosa</i> VRFPA04
<i>P. aeruginosa</i> YL84

Those genes that did not have an ortholog in PAO1 were clustered using CD-HIT [108] , using a threshold of 90% identity and default settings. This resulted in 698,710 gene clusters. Clusters containing fewer than 20 genes were removed. Clusters with 19 or fewer genes, even if exclusively found in CF or non-CF strains, would only have a p value of 5.9×10^{-5} in a Fisher's exact test, which is below the standard significance threshold of a GWAS, 1×10^{-8} . This left 10,082 clusters,

each representing an accessory gene, in addition to 560 accessory genes that were identified in PAO1. Thus, the full pan-genome gene presence/absence dataset consisting of all core (4997) and accessory (10,642) genes, had 15,639 genes (Table 3).

Core genes were aligned against the PAO1 reference, using T-Coffee for translation to amino acid sequences as well as amino acid alignment using default settings. Default settings were used with accurate alignment mode. The number of SNPs for each gene was determined from alignment files. Genes with more than 100 SNPs were manually checked to remove poor alignments. SNPs present in less than 1% of the population were removed, leaving 54,662 SNPs in 4652 core genes to analyze.

Table 3: Description of datasets analyzed and their size.

<i>Dataset</i>	<i>Details</i>	<i>Size</i>
1	Genes in pan-genome	15,639
2	SNPs in core genes	54,662
3	Genes in pan-genome + SNPs in core genes	70,301
4	Genes and SNPs identified with random forest with datasets 1 & 2	561

2.2. Phylogeny

A whole-genome phylogeny was inferred using ExaML [109], a parallelized version of RAxML [110], on the Cipres [111] cluster using 1098 core genes. Only genes recovered in 95% of strains and not found in duplicate in any isolate were used. Gene alignments were performed with T-Coffee [112], using the amino acid sequence and *quickaln* T-Coffee MSA. Reverse translation was also done using T-Coffee, and DNA sequences for the genes were concatenated to create a single multiple sequence alignment. 11 duplicate strains were removed from the dataset; these strains had exactly the same sequence as another strain. The GTRCAT model with 25 categories and 100 bootstraps was used for maximum likelihood inference, along with Stamatakis ascertainment correction.

2.3. fastStructure

FastStructure [54] was performed to infer population structure. FastStructure determines structure within a population using a variational Bayesian framework. Default settings were used, with the number of populations from 1 to 50, inclusive. 13 populations was found to maximize the marginal likelihood, while 29 populations were needed to explain the structure of the dataset. I

investigated the makeup of clusters 13 through to 29 to see how many strains belonged to 0, 1, 2 or 3 populations. A strain was deemed to belong to a single population if it was assigned 60% or more to a population. If there was no population that had an identity of 60% for a strain, then I counted any population with an identity of over 30%. If no populations had an identity of 30% or more, then a strain belonged to 0 populations. Only 13, 24 and 26 clusters had 0 strains that belonged to less than 1 population. 24 and 26 clusters required more groupings of populations to explain the structure, therefore 13 populations was determined to best suit the data.

For determining the cluster a particular strain belonged to for analysis purposes, only associations greater than 66% were assigned. If a strain belonged to a cluster with less than 66% association, they were assigned no cluster.

2.4. Association Tests

2.4.1 PLINK

PLINK version 1.9 [48, 49] was used for stratified case/control association, combined with the cluster information from fastStructure. PLINK is often used for human GWAS studies, and has been used in a few bacterial GWAS [61, 79, 78]. PLINK is a whole pipeline that can filter SNP files and determine genetic associations with various tools and algorithms. Default settings were used. LD was also estimated with PLINK, as r^2 values between SNPs or genes. PLINK was performed on all datasets.

2.4.2 treeWAS

TreeWAS [67] is a new program that uses phylogeny to infer genetic associations with a given phenotype. It was created with microbial GWAS in mind, and tries to eliminate the confounding effects of areas in high linkage disequilibrium in the genome, as well as population structure. It has three ways of doing this, though a terminal test, simultaneous test, and subsequent test. The terminal analysis does not require reconstruction of ancestral nodes in the phylogeny, but rather relies on the terminal state. The simultaneous analysis looks at the probability of changing phenotype and genotype states along a branch, is not impacted by missing associations, and can infer relationships involving multiple changes along a branch. The subsequent analysis calculates the probability of finding the same genotype and phenotype on each branch.

Association scores are reported for each analysis. These association scores are calculated by first creating a null distribution of association scores using a simulated dataset, where there is no association between a loci and the phenotype. The association between each loci is then measured and calculated, following the same distribution of the null association scores. Scores and loci at the upper end of the null distribution are reported.

TreeWAS was used with each dataset and one of the bootstrap phylogenies created with ExaML. Strains that had been removed by ExaML due to sequence similarity were re-introduced to the tree, sharing a node with the strain their sequence perfectly identified with.

2.4.3 Machine Learning Methods

Random forest (RF) and support vector machine (SVM) classifiers were performed on each dataset of 1586 *P. aeruginosa* strains, using the *scikit-learn* package in Python 3.6. Random forests were run with 10 decision trees, and the maximum possible number of features for each dataset. Support vector machines were run with a linear kernel.

2.4.4 Other Statistical Tests

Fisher's exact test and principal component (PC) analysis were carried out using Python package *scikit-learn*. All boxplot comparisons of PCs were output and analyzed. Only PCs with significant differences between CF and non-CF strains were plotted against each other.

3. DATASET RESULTS

If a gene was in 95% of strains, it was identified as a core gene (Figure 4) resulting in a core genome dataset of 4997 genes. Strains missing more than 10% of core genes were removed from the dataset. This left 1586 strains for analysis.

Figure 4: Number of PAO1 genes present in proportion of strains in the dataset. A 95% threshold was used for the core genome - if a gene was in 95% or more of strains, it was labelled as a core gene. 4997 genes were part of the core genome.

3.1. Phylogeny

I inferred a maximum-likelihood phylogeny for all 1586 genomes using ExaML [109]. CF and non-CF strains are interspersed throughout the tree. This implies that the CF strains do not all have shared ancestry, and that CF infections represent multiple, independent evolutionary events (Figure 5). Furthermore, for most studies that contributed more than one strain, strains are spread out around the tree (studies that contributed over 100 strains and that were not found in a single clade are highlighted). Only 2 studies that contributed more than 40 strains were found to be in a monophyletic clade. The leaves highlighted in green are from a study of the Liverpool epidemic strain. The other highlighted leaves, in orange and pink, were from a longitudinal studies of PA samples from single patients, one in Toronto (orange) [16], the other in Italy (pink) [17]. This phylogeny was subsequently used to ensure that positive associations were not due to shared ancestry alone, and to analyze the datasets using a phylogeny-based GWAS method, treeWAS [67].

3.2. Population structure

Population structure may confound GWAS analyses: if affected individuals tend to be drawn from the same population, then non-causal variants that happen to occur at a higher frequency in that population may falsely be associated with the disease. As such, I used FastStructure [54] to infer population structure in this dataset.

Cluster proportion identified from FastStructure can be seen in Figure 6. There are three clusters of just CF strains, one of which contains 220 strains which were all collected from a single patient. Three more clusters are composed of 95% or more CF strains: one of them contains all of the Liverpool epidemic strains, along with one non-CF strain. Two clusters are a 35/65 split of CF to non-CF strains. Only one cluster is 95% or more non-CF strains. One cluster has no members that are associated with it fully. 436 strains (287 non-CF, 149 CF) are not assigned to a cluster with 66% or more membership.

Phylogenetically, the FastStructure clusters are not monophyletic, but several of the clusters have all members in close proximity within the tree.

Figure 5: Phylogeny of 1576 *P. aeruginosa* genomes. Red squares outside the tree denote CF strains. Studies contributing >100 strains are highlighted (red and blue), as are studies with >40 strains found in a single clade (orange, pink and green). Branch colours show bootstrapping confidence, green is 100, red is 1.

4. GENE PRESENCE/ABSENCE DATASET RESULTS AND DISCUSSION

4.1. Linkage Disequilibrium

In addition to population structure, high linkage disequilibrium can confound association results. I examined LD in the gene presence/absence dataset (Figure 7) using PLINK. Aside from a few short sections, there is little linkage between genes. In *P. aeruginosa* there is little LD beyond 3kb, and there is a rapid decrease in LD after about 200bp [113]. Since most genes in the accessory genome are separated by more than 1kb, this explains why the figure shows little LD.

4.2. PLINK

I carried out a case-control association analysis in PLINK, using 13 population clusters to attempt to control for population structure. A quantile-quantile (QQ) plot was generated as a quality control measure. A QQ plot shows the distribution of calculated p values against a uniform distribution of expected p values. The QQ plot (Figure 8) shows that the calculated p values are substantially higher than the expected p values. This suggests that there are many type I errors (false positives) in the PLINK results. Ideally, we would want to see the datapoints mostly follow the red line (which represents a uniform distribution of p values), with a deviation near the tail where there are more significant associations. There could be some population structure not accounted for by fastStructure, which is often the case in human GWAS with QQ plots that show an excess of type I errors.

Even though the QQ-plot is not what I was ideally looking for, the PLINK association tests gave interesting results. It identified 145 genes as contributing to a CF phenotype, over 13 clusters or families (genome significance cutoff of 1×10^{-8}).

Figure 9 shows a Manhattan plot of the genes tested in PLINK, and is usually a way to visualize significant genes in a study. In an ideal plot, "spikes" of points will appear, with some of the points in these spikes above the significance threshold. What this indicates, biologically, is that there is a gene/SNP at this location that is associated with the phenotype studied. Although there will be often be several points above the significance threshold in a given spike, some of these other points may appear to be associated with the phenotype due to high LD between it and a causal variant. It is the researcher's job to try to keep this in mind, and try to identify the causal variant

Figure 6: Proportion of cluster association for all strains. The x-axis is the strain, ordered by population. The y-axis is the proportion of belonging to a cluster.

Figure 7: Linkage disequilibrium pattern in *P. aeruginosa* genes, r^2 as calculated in PLINK. The x-axis represents the location along the pan-genome. The first 3513 locations are ordered as in strains from Table 4. The last 6000 positions are ordered as found in PAO1.

Figure 8: QQ plot of calculated $\log p$ values in PLINK of the gene dataset against expected p values. The red line is when the observed p values equal the expected p values ($x=y$).

Figure 9: Manhattan plot of p values in the PLINK analysis of the gene dataset. Points above the red line are significant, and coloured in green. The red line is the genome-wide significance line, at $\log(1 \times 10^{-8})$. The blue line is the suggestive significance line, at $\log(1 \times 10^{-5})$.

from the non-causal variants in these spikes. The difference between Figure 9 and a Manhattan plot from a human GWAS study is that all genes are in the same order in humans, and so the plot would ideally show spikes in certain areas (resembling a Manhattan skyline, hence the name). However, in bacteria, genes are in different orders in each strain, so a standard sequence and spikes in certain areas can be unattainable. Any points above the line represent genes with significant associations (in green). Although the order for the first 3000 genes are as they are found in a single strain (Table 4), and the last 5000 genes are ordered as in PAO1, there is little linkage between genes with significant associations. This is due to a lack of LD, since I found little long distance LD in the dataset (Figure 7) - there are few genes that are found in combination with each other consistently, no more than if random assortment were to occur.

Table 4: Strains used for ordering of accessory genes. Only reference genomes with known orthologs to PAO1 that had 100 or more unaccounted for accessory genes were used. Ortholog information was retrieved from the online *Pseudomonas* database [1]

<i>Strain</i>	<i>Number of accessory genes</i>
NCGM1984	768
VRFP404	517
LES400	457
19BR	436
PA7	286
SCV20265	239
PACS2	231
UCBPP-PA14	180
PA1R	153
F22031	140
MTB-1	106

Many of top genes found by the PLINK association tests are hypothetical proteins or pseudo-genes. The characterized genes in the top 40 results are listed in Table 5. Several of these genes have known functions.

tonB genes encode for outer membrane receptor proteins that are involved in iron-acquisition. *tonB2* was the second iron acquisition gene found in the PA genome. In recent years, iron acquisition has been shown to play an important role in bacterial CF phenotypes. Ferrous iron is abundant in CF sputum [114]. There is controversy over whether virulence of PA is impacted in an iron-rich or iron-poor environment, and the amount of iron in a CF lung environment [114, 115, 116, 117, 118, 119]. Furthermore, *tonB2* was found to be a determinant for gentamycin resistance in a small-scale GWAS that looked for associations between genes and antimicrobial resistance [107]. Iron transport

Table 5: Top genes from from PLINK stratified association analysis

<i>Rank</i>	<i>Gene</i>	<i>p value</i>
1	<i>tonB2</i>	3.09×10^{-33}
5	<i>recF</i>	1.66×10^{-20}
12	two-component response regulator	1.63×10^{-18}
16	<i>pdtA</i>	4.08×10^{-18}
17	<i>hxcY</i>	6.46×10^{-18}
20	<i>napA</i>	2.62×10^{-17}
24	<i>mexZ</i>	6.61×10^{-16}
25	<i>oprB</i> precursor	1.38×10^{-15}
30	<i>mucA</i>	5.73×10^{-15}
31	<i>mutL</i>	7.61×10^{-15}
33	<i>mexB</i>	3.61×10^{-14}
36	enolase-phosphatase E-1	7.61×10^{-14}

systems have been looked at as a possible method to tackle antibiotic resistance [118], something which is abundant in *P. aeruginosa* infections in CF patients. Looking at the dataset, a large majority of CF strains are missing *tonB2* (768/947 missing, over 80% of CF strains), while only about half are missing this gene in non-CF strains (347/639).

RecF is a non-essential DNA replication and repair protein that has been found to be involved in recombination, but has not been linked to a CF phenotype. It has, however, been shown to have some importance in Ciprofloxacin resistance in a small study that used a random forest [107], although the authors readily admit that their results are questionable due to such small training sets. *recF* is overwhelmingly found in strains that are not from a CF source (578/639, 90% of non-CF strains), but presence and absence of the gene is equally split among CF strains (444/947 contain the gene, or 47%).

pdtA encodes for phosphate depletion regulated TPS partner A, a protein involved in a two-partner secretion system that increases virulence of PA strains [120, 121]. Limiting inorganic phosphate activates the expression of *pdtA*. The mechanism of increasing virulence is unknown, but it was demonstrated that *pdtA* mutants are less virulent than wild-type strains with a functional *pdtA* in a *C. elegans* killing assay [120]. *pdtA* was present in only 85% of CF strains (812/947), but 97% of non-CF isolates (623/639).

hxcY encodes a type II secretion system protein, but its function has not yet been researched. *napA* is a periplasmic nitrate reductase protein which has been found to be upregulated in Liverpool epidemic strains when compared with PAO1 [122] and could be a possible immunogenic protein

[123].

Multidrug efflux pumps (Mex proteins) confer antibiotic resistance by pumping out drugs and other materials from the cell [124]. The role of efflux pumps in antibiotic resistance in CF PA strains has been widely studied [30, 125, 126]. *mexZ* encodes for a well characterized protein that confers aminoglycoside and fluoroquinolone resistance [127, 128, 129]. MexZ regulates the MexXY multi-efflux pump, and is often found mutated in *P. aeruginosa* coming from CF lungs. In the dataset, the gene is missing in more CF strains than not (missing in 749/947 strains, 79%), while it is more present than not in non-CF strains (missing in only 249/639 strains, 39%). The absence of MexZ would leave MexXY unrepressed, increasing the bacterium's resistance to antibiotics by more effectively pumping out drugs. *mexB* encodes for a different efflux pump that confers resistance to beta-lactams, tetracyclines, trimethoprim, and fluoroquinolones [130]. Similar to *mexZ*, *mexB* was absent more often in CF strains (missing in 80/947, 8.4%, missing in only 14 non-CF strains, 2.2%), however the discrepancy between CF and non-CF strains was not as large. It is not immediately clear why *mexB* might be more often absent in CF strains, as it encodes for an efflux pump; it is not just a regulator like MexZ.

The outer membrane porin protein OprB has been characterized as a carbohydrate transfer protein in PA [131]. When combined with a knockout of *bpeAB* in *Burkholderia pseudomallei*, however, it confers broad spectrum drug resistance [132, 133, 134]. *bpeAB* have not been found in PA, and this link between *oprB* and antibiotic resistance has not been yet been demonstrated in the species. It is however possible that a similar mechanism of action exists in PA. Alternatively, since the CF lung presents a unique environment for PA, it is possible that *oprB* is not as useful for carbohydrate transfer as in other environments. Looking at the correlation between the presence/absence of the gene in CF and non-CF strains, the gene is missing in roughly a third of CF strains (329/947), but is only missing in 14% of non-CF strains (92/639).

Deletion of *mucA*, a gene identified as contributing to the CF phenotype with PLINK association analysis, has been shown cause mucoidy in *P. aeruginosa* [15, 135]. A mucoid characteristic is common in CF infections. MucA is part of the AlgU pathway, which encodes RNA polymerase sigma-H factor. When the *algU* pathway is dysfunctional (either due to missing or defective *algU*, *algD* or *mucA* genes), the strain becomes mucoid [135, 136]. This helps the strain become established in the lung and adapt to the host's stress response [6]. Looking at its occurrence in CF and non-CF

strains, the gene is missing in more than half of CF strains (only 431/947 strains have the gene, 45.5%), while only 45 non-CF strains were missing the gene (gene is present in 594/639 non-CF strains, 93.0%). This could be because the absence of *mucA* in a non-CF strain leads to a substantial loss of fitness in other environments.

mutL encodes a protein that is part of the mismatch repair system. Issues with DNA repair can lead to more mutations within the genome and increase the rate of adaptation. MutL loss-of-function in bacteria has been shown to promote large-scale chromosomal deletions through double strand breaks and promote hypermutation [137, 138]. *mutL* was present in 887/947 CF strains (93.7%), and almost all non-CF isolates (634/639, 99.2%).

4.3. treeWAS

TreeWAS scores genes based on their association to a phenotype while avoiding clonal associations (associations based on identity by descent). It corrects for population structure differently than PLINK, using the phylogeny and phenotype to look for significant gene associations that are not confounded by shared heredity. It incorporates different models, calculating associations when there are phenotypic state changes along branches, or not. With the simultaneous model (ie. changes in genotype and phenotype were allowed along a branch), association scores with an absolute value larger than 19 were deemed significant, identifying 129 genes of interest (Table 6, Figure 10). Well over half of the genes (74/129) were hypothetical proteins, however, and many of the top genes were unexpected from previous research into infectious PA strains. The top genes that have known functions were also found and described in the PLINK section (Section 4.2), such as *tonB2* and *recF*.

Table 6: Top genes found in treeWAS combined association analysis.

<i>Rank</i>	<i>Gene</i>	<i>Association Score</i>
1	protease	−50
2	<i>hxcY</i>	−49
3	PA1539	−49
4	PA1729	−48
5	<i>napA</i>	−46
7	<i>tonB2</i>	−45
9	<i>recF</i>	−43
13	PA2074	37

Figure 10: Manhattan plot of gene scores found from treeWAS association simultaneous analysis.

4.4. Random Forest

Random forests create a set of decision trees that look to accurately predict the phenotype using as few variants as possible. They use the information from these decision trees to create an overall importance metric, which identifies the variants that are the best predictors of the phenotype. The model had $93.4\% \pm 1.0\%$ accuracy when predicting CF versus non-CF strains when given presence/absence information for about 265 genes (Table 7). This means that knowing the presence or absence of 265 genes, the model could correctly predict a whether a strain was CF or non-CF 93.4 out of 100 times. Several of the top genes identified by random forest are already corroborated in literature, lending support to the model. Many of the top genes were also found in the PLINK and treeWAS tests (Sections 4.2 & 4.3). The top hit from random forest, PA2074 (unknown function) was also identified in the treeWAS analysis in the top 15 genes.

Table 7: Top genes that contribute to the random forest model.

<i>Rank</i>	<i>Gene</i>	<i>Importance</i>
1	PA2074	0.237
2	<i>mucA</i>	0.123
3	<i>tonB2</i>	0.084
4	PA5037	0.041
5	<i>recF</i>	0.029
8	<i>mexF</i>	0.011
10	efflux pump	0.009
11	<i>mutL</i> pseudogene	0.009

The random forest approach identified *mucA* as being important to the CF-phenotype within the top 5 genes, which was not the case with PLINK nor treeWAS. PLINK did identify *mucA* as the 30th most important gene contributing to a CF phenotype, while treeWAS did not identify it at all.

mexF is a gene that encodes for an efflux pump and contributes to antimicrobial resistance in various bacterial strains, similar to *mexB* and *mexZ* described in section 4.2. *mexF* was present in a higher proportion of non-CF strains compared to CF strains (present in only 66%, 626/947, of CF strains; present in 98% non-CF strains, 626/639).

Other named genes with known functions that contributed to the RF model were identified in the earlier PLINK section (Section 4.2).

I carried out several additional tests with RF to assess the effects of potential confounding factors. For example, genes were dropped that were identified in only one study. Only 77 genes fit this

criteria, none of which were found to be important in the original model, and removing them did not change the results. In another test, genes that had a presence/absence scheme that was 95% similar were collapsed to a single gene cluster. As such, all core and a few accessory genes (5096 genes total) were combined into 1 cluster. This new model needed only 148 genes to determine the difference between CF and non-CF strains, and did so with $93.8\% \pm 1.0\%$ accuracy. 46 genes identified in the first RF model were combined into the 'core' cluster, and therefore did not appear in the new model. 97 genes were identified that were not in the first model, and 51 genes appeared in both models (Appendix C.1).

4.5. Support vector machines

An SVM was able to separate the CF from the non-CF strains with 94.0% accuracy. Unlike with an RF, an SVM does not output a smaller set of important genes/features, but rather every gene has a coefficient in the hyperplane, and it is up to the researcher to determine what the cut-off is. A strongly negative or positive coefficient for the hyperplane contributes more to the model than values that are close to 0. As can be seen in Figure 11, the distribution of coefficients resembles a log-odds curve when values are plotted from lowest to highest. Most coefficients are around 0, with only 510 genes lower than -0.02 or higher than 0.02. Of the genes with the highest absolute hyperplane coefficient, most of the top six genes are recognizable and expected (Table 8). *mucA* and *tonB2* were among the most important genes identified in the RF model as well.

The phenazine biosynthesis protein PhzE is a catalyst for synthesizing virulence factors, such as pyocyanin and phenazines in *P. aeruginosa* [139]. It has been shown to be overexpressed in burn victims and underexpressed when grown in the blood of healthy patients (when compared to growth in LB broth) [140]. In CF patients, strains that produce auto-antibodies against bactericidal/permeability increasing protein (BPI-ANCA) cause infections that are more difficult to treat. These strains are found to produce fewer phenazine derivatives. Strains without BPI-ANCA, in vitro, produced more phenazine derivatives, and induced a higher immune response in epithelial cells [141]. In this dataset, the gene is more often absent in CF strains (only present in 349/947 strains).

Unfortunately, after the top six genes, many of the genes with high absolute coefficients are hypothetical proteins or do not stand out as contributing to a CF phenotype. 196 out of 504

remaining genes with absolute hyperplane coefficients greater than 0.02 encode for hypothetical proteins, without counting pseudogenes.

Table 8: Top genes found in support vector machine model.

<i>Rank</i>	<i>Gene</i>	<i>Absolute hyperplane coefficient</i>
1	<i>mucA</i>	0.089 61
2	<i>oprB precursor</i>	0.0875
3	<i>mexZ</i>	0.0727
4	<i>phzE</i>	0.0654
5	PA2727	0.0619
6	<i>tonB2</i>	0.0584

4.6. Fisher's exact test

I performed a Fisher's exact test on each gene, as this is a common statistical method used in other GWAS [142]. A Fisher's exact test simply asks whether a gene is more often present (or absent) in CF vs. non-CF isolates. One challenge in performing a Fisher's exact test is determining the significance threshold. Over 1000 genes had p values less than 1×10^{-20} , and around 200 genes had odds ratios of infinity (meaning that given the presence of that gene, there was an infinite chance the strain was from a CF patient). Given such low p values, even after a Bonferroni correction (p value significance cutoff is divided by the number of variables, so any p value $< 3.2 \times 10^{-6}$ is deemed significant), thousands of genes were over- or under-represented amongst CF strains of PA. The Manhattan plot shows thousands of genes to be significant (Figure 12); 1000 genes is too many to test in a lab in a reasonable amount of time. The odds-ratio was also unhelpful, as many of the top genes were only observed in one study, which suggests that the results are due to artifacts from sequencing or proximity of strains and are not due to the strain adapting to a cystic fibrosis lung environment. Without more samples, the Fisher's exact test is unable to provide valuable insights. Furthermore, with a standard Fisher's exact test, I am not able to correct for population structure, something that other GWAS studies do, which could make fewer genes significant to a CF strain of PA.

4.7. Principal component analysis

Principal component analysis (PCA) is a method of dimensionality reduction whereby many dimensions and the data they describe are "summarized" by a smaller number of vectors which

Figure 11: Hyperplane coefficient from linear support vector machines. Gene index was sorted based on lowest SVM coefficient to highest, to show the spread of values.

Figure 12: Manhattan plot of p values from a Fisher's exact test for each gene found in any of the 1600 tested *P. aeruginosa* strains. The red line is the genome-wide significance line, at $\log(1 \times 10^{-8})$. The blue line is the suggestive significance line, at $\log(1 \times 10^{-5})$.

capture the greatest differences in those feature dimensions and thus reduce the number of dimensions. This is performed at the cost of losing those minimal differences between dimensions and operating in a new, smaller feature space that no longer contains exactly the original dimensions. PCA was performed on the dataset to see if, after a reduction in features, there were any components that separated the CF strains from the non-CF strains. There were 54 components that accounted for 50% of the variance between different strains. PC 1 did not differentiate CF from non-CF strains, however PCs 2 and 3 did (Figure 13). A plot of PC2 against PC1 (Figure 14) was able to show two clusters, one that was primarily CF strains (orange), and one that was mostly non-CF strains (blue).

The primary genes that make up principal components 2 and 3 are hypothetical proteins. None of the top 10 genes in either component (either strongly positively or negatively correlated with the component) are strongly supported in the literature. Genes that would be expected to be of importance (antibiotic resistance genes, metabolism or mucoidy genes) contribute little to the components. These results could be taken into account when looking at other machine learning results, however they are not promising on their own.

4.8. Conclusion of gene presence/absence dataset

There are some genes that were found consistently using PLINK, treeWAS, RF and SVM (Figure 15). *tonB2* was recovered using each of the four methods, within the top 10 genes identified. *hxcY* was also found in each of the methods, however was not strongly significant in three of the four methods. A few hypothetical proteins were also identified with these four methods: PA5037, an unnamed non-ribosomal peptide synthetase, and an undefined hypothetical protein.

Some of the genes highly cited in literature were identified in three of four methods, such as *mucA*, *mexB* and *mexZ*, *mutL*, and *recF* (Figure 16). The top genes identified using a random forest had the fewest hypothetical and most corroborated genes in literature. Though this model found the largest number of genes as contributing to a CF-phenotype, most of these genes had little importance (0.001), and could be excluded when identifying which genes should be further researched.

Figure 13: Boxplot of mean principal component values of genes for cystic fibrosis and non-cystic fibrosis strains. CF_flag of 1 is a cystic fibrosis strain, CF_flag of 0 is a non-cystic fibrosis strain. A) Principal component 2 mean values, B) principal component 3 mean values.

Figure 14: Plot of principal component 2 values against principal component 1 for genes of cystic fibrosis and non-cystic fibrosis *P. aeruginosa* strains.

5. MUTATION DATASET RESULTS AND DISCUSSION

5.1. Linkage Disequilibrium

LD was tested with the SNP dataset using PLINK, using default settings. LD was higher between SNPs within the core genome than between genes in the pangenome (Figure 17). LD drops off rapidly, within the first few kilobases (Figure 18), which is consistent with previous research [113]. The hotspot of LD about halfway in the genome includes several metabolism and transport genes, such as *pvdL* and *mexE*, along with many unclassified genes (80 of 111 genes in that area are hypothetical). The highest mean r^2 value was around 500bp, at 0.7655. A 50% reduction in this LD was found after just 700bp, with an r^2 value of 0.3811.

5.2. PLINK

I carried out a PLINK case/control association study for the SNP dataset using the populations from fastStructure with the gene dataset. This gave a QQ plot with the lowest deviation from the expected p values. The QQ plot (Figure 19) with the SNP dataset shows a larger deviation from the expected p values than the gene dataset (Figure 8). Nonetheless, there is still a marked deviation from the expected uniform distribution.

The Manhattan plot (Figure 20) does not have spikes, as might be expected from a human GWAS Manhattan plot. There are many SNPs that were identified in PLINK as contributing to a CF lung environment adaptation.

Associations identified in the PLINK analysis include SNPs in several genes with known or suspected roles in adaptation to the CF lung, while others are not known to be involved in CF pathoadaptation (Table 9). For example, the ATP-binding component of an ABC transporter has been suspected to contribute to ciprofloxacin (antibiotic) resistance [143]. The deletion at position 269 was in only 320/947 CF strains (33.8%), but 400/639 non-CF strains (62.6%).

PqqF is a required protein for PQQ (pyroquinolone quinone) biosynthesis. The elimination of pqqF results in the inability of the bacterium to produce prodigiosin from glucose [144, 145]. The H12R mutation is present in 80% of CF strains (767/947), while it is only present in 26% of non-CF strains (170/639). The H3R mutation has similar statistics, present in 761/947 CF strains, but only 165/639 non-CF strains. The r^2 value between these two mutations is 0.965, meaning I

Figure 17: Linkage disequilibrium pattern in *P. aeruginosa* core genome mutations, as calculated in PLINK. The x-axis represents the location along the PAO1 genome.

Figure 18: Linkage disequilibrium (r^2) decay in *P. aeruginosa*.

Figure 19: QQ plot of calculated $\log p$ values in PLINK of the mutation dataset against expected p values. The red line represents when observed p values equal the expected p values ($x=y$)

Figure 20: Manhattan plot of p values in the PLINK analysis of the SNP dataset. Points above the red line are significant, and coloured in green. The red line depicts the genome-wide significance line, at $\log(1 \times 10^{-8})$. The blue line represents the suggestive significance line, at $\log(1 \times 10^{-5})$.

Table 9: Top SNPs and indels from from PLINK stratified association analysis

<i>Rank</i>	<i>Gene</i>	<i>Mutation</i>	<i>p value</i>
1	probable ATP-binding component of ABC transporter	269 deletion	3.32×10^{-27}
6	<i>pqqF</i>	H12R	4.31×10^{-21}
8	<i>pqqF</i>	H3R	6.54×10^{-18}
10	<i>proA</i>	R275Q	6.07×10^{-14}
12, 13	<i>wapB</i>	D12S, S24A	3.97×10^{-13}
15	<i>narG</i>	A144V	1.26×10^{-12}
16	<i>cysH</i>	L2P	2.54×10^{-12}
19	<i>iciA</i>	M1L	2.51×10^{-11}

cannot determine which mutation is causal since they are in high linkage disequilibrium.

ProA is a gamma-glutamyl phosphate reductase that is involved in proline biosynthesis. There is no research to show that this is involved in the adaptation to a CF lung environment or that it contributes to antibiotic resistance. The mutation at position 275 is more common in non-CF isolates (present in 167/947 CF strains, 17.6%, and 259/639 non-CF strains, 40.5%).

wapB is a newly characterized gene in PA. It is involved in the outer core biosynthesis of lipopolysaccharide (LPS) in the cell membrane [146, 147]. The LPS is a major virulence factor that affects the inflammation and immune response of the host [148]. Both mutations have similar prevalence among CF and non-CF strains. D12S is present in 263/947 CF strains (27.8%), while S24A was found in 271/947 (28.6%). Both mutations were in 9/631 non-CF strains (<1%). The SNPs are in high linkage disequilibrium, with an r^2 of 0.838; only one strain containing D12S did not also have the S24A mutation, while S24A was found 9 times without D12S. As with the mutations in *pqqF*, I cannot determine if one or both of the mutations are causal as they are in high linkage disequilibrium.

narG encodes for a respiratory nitrate reductase alpha chain. It is responsible for the anaerobic respiration of PA [149, 150, 151]. A144V is not present in many CF or non-CF strains. It was only found in 86/947 CF isolates (9.1%), and 13/639 non-CF (2%).

CysH is a 3'-phosphoadenosine-5'-phosphosulfate reductase identified as being involved in biofilm formation [152]. The mutation L2P is present in almost 50% of CF strains (456/947) but only 6% of non-CF strains (39/639).

IciA is an inhibitor for chromosome replication initiation that has been studied in *E. coli*. If it is highly expressed, there is a longer lag time when bacteria are introduced to a new media [153]. It has not been linked to CF adaptation or virulence at this time. The M1L mutation was present in

50% of CF strains (467/947), but only 5% of non-CF strains (33/639).

5.3. treeWAS

TreeWAS gave less corroborated results for the SNP dataset. Few of the most important genes identified were found with other methods or were recognizable in literature. 193 SNPs were found in 44 genes in the simultaneous analysis, most of which were hypothetical. Most of the named genes found in Table 10 were described in Section 5.2. Given the spikes in the treeWAS Manhattan plot (Figure 21), several of the SNPs identified are in LD, meaning that not all of them may actually contribute to adapting to a CF-lung environment, but rather that they are inherited together due to their proximity on the chromosome. The top two mutations in *pqqF* are a good example of this. The spike just after position 30,000 in Figure 21 represents a number of SNPs found in just one gene, encoding hypothetical protein PA3290.

Table 10: Top SNPs and indels found in treeWAS combined association analysis.

<i>Rank</i>	<i>Gene</i>	<i>Mutation</i>	<i>Association Score</i>
1	<i>pqqF</i>	H3R	51
2	<i>pqqF</i>	H12R	50
3	<i>tle1</i>	D168E	45
4	<i>iciA</i>	M1L	44
5	PA5270	S5A	43
6,7	PA1355	F33S, C37G	42

tle1 was not identified in the PLINK analysis. It is a type VI secretion phospholipase effector that targets and hydrolyzes membrane phospholipids. It is part of the T6SS, which has antibacterial properties [154]. In *K. pneumoniae*, T6SS has been induced by sublethal antibiotic concentrations (tested with beta-lactams and apramycin), and activation of *tle1* may make isolates more lethal to other bacteria present [155].

5.4. Random forest

The random forest model had 92.5% accuracy and identified 296 SNPs needed to predict the phenotype of an isolate. Many of the genes identified were hypothetical or found through other analyses.

Several of the mutations found with a random forest were identified through PLINK and treeWAS (Table 11). The functions of *pqqF* and *cysH* were previously discussed in the PLINK section.

Figure 21: Manhattan plot of SNP scores found from treeWAS association simultaneous analysis.

K92R in *pqqF*, a mutation not identified in PLINK, was present in over 90% of CF strains (865/947), and almost 95% of non-CF strains (607/639), but it is unclear how this mutation affects the function of the protein. *wbpM* is involved in the O side chain synthesis of the LPS and makes the O-antigen more temperature dependent [156]. Mutations to this gene can change the stability of the O-antigen which can adapt and avoid immune responses from the host. If the gene is knocked out, there is no O side chain produced in the LPS [157]. The V113P mutation was present in only 4.8% of CF strains (46/947), and over 40% of non-CF strains (278/639). YecS is an L-cystein transporter of the ABC system that is involved in host adaptation. It has not been well characterized, but studies have linked it to being involved with CF strains of PA [158, 23]. In this dataset, an insertion at position 165 was only found in CF strains (252/947 CF strains have the mutation, but only 1 non-CF strain has been found with the mutation).

Table 11: Top SNPs and indels that contribute to the random forest model.

<i>Rank</i>	<i>Gene</i>	<i>Mutation</i>	<i>Importance</i>
1	<i>pqqF</i>	H12R	0.14
2	<i>pqqF</i>	H3R	0.098
4	<i>wbpM</i>	V113P	0.049
6	<i>pqqF</i>	K92R	0.039
8	<i>iciA</i>	M1L	0.012
9	<i>cysH</i>	L2P	0.012
15	<i>yecS</i>	164 insertion	0.008

5.5. Support vector machine

SVM had 89.9% accuracy with the core SNP dataset. Several of the mutations identified were also recovered in PLINK, treeWAS or the random forest approach (Table 12).

Table 12: Top SNPs and indels found in support vector machine model.

<i>Rank</i>	<i>Gene</i>	<i>Mutation</i>	<i>Absolute hyperplane coefficient</i>
1-4	dipeptidase	21 insertion, G22A, G22V, 22 insertion	0.5
5	<i>pirA</i>	A370T	0.339
6	<i>mexK</i>	E765A	0.337
7	<i>mexK</i>	K694R	0.291
8, 9	<i>pirA</i>	A228V, V128A	0.281
10	<i>mutL</i>	E428D	0.281
15, 16	<i>wbpM</i>	I7G, K8R	0.19
18	<i>gyrA</i>	D87Y	0.176
20	<i>yecS</i>	164 insertion	0.151

PirA is necessary for the action of siderophore antimicrobial drugs, such as cefiderocol, a catechol conjugate, and MC-1, a monocarbam. Without this receptor, siderophores lose their mechanism of action [116, 159, 160]. The A370T *pirA* mutation is mostly present in the PA strains studied (913/947 CF, 624/639 non-CF). The other two *pirA* mutations in Table 12 are absent in most strains (A228V is present in 23/947 CF and 6/639 non-Cf strains, V128A is present in 29/947 CF, 9/631 non-CF). *mexK* can affect antimicrobial resistance as well, as it encodes for another efflux pump as described in earlier sections. The E765A mutation is present in 65/947 CF strains, and only 18/639 non-CF strains. The K694R mutation has a higher prevalence, found in 30% of CF strains (283/947) and 44% of non-CF strains (282/639).

mutL was discussed in the gene presence/absence dataset, (Section 4), as it was missing more often in CF strains than non-CF strains and identified as being important to the CF phenotype. It is involved in DNA repair and loss-of-function can create hypermutator strains. It was present in enough genomes to still constitute a core gene. The mutation E428D is present in few strains: 12/947 CF and 28/639 non-CF.

gyrA encodes the gyrase subunit A protein. The D87Y mutation has been characterized in other studies to contribute to resistance to antimicrobial drugs, namely fluoroquinolones [32, 33, 161]. The D87Y mutation was present in 29/947 CF strains, and only 9/639 non-CF strains, as might be expected in CF patients treated with quinolones.

5.6. Fisher's exact test

As with the Fisher's exact test done using the gene dataset, thousands of genes had p values less than 1×10^{-20} , which is significantly smaller than the Bonferroni cutoff of 1×10^{-8} . The Manhattan plot for the Fisher's exact test (Figure 22) of this dataset shows a few more peaks than the one from section 4, however the lack of LD is still evident in the significance of SNPs located close together on the chromosome.

5.7. Principal component analysis

Principal component 1 splits the CF strains of PA from non-CF strains the best (Figure 23). PC1 accounts for 19.2% of the variance, while PC2 only accounts for 9.8%. The model required 9 components to account for 50% of the variance in the dataset and, aside from component 1, none

could split the CF from the non-CF strains (Figure 24).

None of the top genes that contribute to PC1 are identified in literature for a CF or AMR phenotype, nor were they found using other analysis methods, aside from perhaps *secF*. SecF is a membrane protein that may be up-regulated in biofilms [162]. There were three *secF* mutations identified in PC1, A109T, D219S and V221I. These mutations were all present in 169/947 CF strains, and between 239 and 244 of 639 non-CF strains.

Figure 22: Manhattan plot of p values from a Fisher's exact test for each SNP found in a core gene. The red line is the genome-wide significance line, at $\log(1 \times 10^{-8})$. The blue line is the suggestive significance line, at $\log(1 \times 10^{-5})$.

Figure 23: Boxplot of mean principal component values of mutations for cystic fibrosis and non-cystic fibrosis strains. CF_flag of 1 is a cystic fibrosis strain, CF_flag of 0 is a non-cystic fibrosis strain. A) Principal component 2 mean values, B) principal component 3 mean values.

Figure 24: Plot of principal component 2 values against principal component 1 for mutations of cystic fibrosis and non-cystic fibrosis *P. aeruginosa* strains.

5.8. Conclusion of core SNP dataset

I examined associations for the mutation dataset, consisting of SNPs or indels (insertion/deletions) within 4653 core genes. Some genes were excluded from the original "core" genome due to poor alignment. This could be corrected for by removing BLAST matches that have a lower threshold in common with the reference, but risks removing genes that are highly similar but short in length. One remedy for this would be using a program such as SuperCRUNCH [163] to find better matches for sequences.

A number of SNPs were found in common between the various methods. Many of these variants were in hypothetical proteins (Figure 25). Two mutations in *pqqF*, H3R and H12R, were identified with all four methods, although they were less significant in the SVM model compared to the other three analyses (Figure 26). Mutations in *iciA* and *cysH* were also recovered in all four analyses.

The SVM identified the largest number of mutations within genes that have known functions. At least one of these mutations is already in literature as contributing to quinolone resistance (D87Y in *gyrA*). Many genes identified in other methods were also recovered in the SVM analysis (Figure 26), although with a lower ranking in SVM compared to the other methods.

Figure 25: Venn diagram of results from PLINK, treeWAS, random forest and support vector machine. SNPs identified in at least 3 of the 4 methods are reported, if there were less than 10.

Figure 26: Heatmap of SNPs found in the core SNPs dataset with various methods. Darker blue is a higher ranking (lower p value or higher association score/ importance/ hyperplane coefficient) variant. Yellow is a lower ranking variant. White is not identified as contributing to the phenotype.

6. GENE AND MUTATION DATASET RESULTS AND DISCUSSION

This dataset was created by combining the gene and mutation datasets (Sections 4 and 5).

6.1. PLINK

The QQ plot from the PLINK analysis of the full dataset has a similar structure to those found with the separated gene and SNP sets (Figure 27), suggesting that some non-causal variants are confounding the PLINK results. The Manhattan plot (Figure 28) shows that many genes and SNPs were identified as contributing to a CF phenotype in the PLINK analysis. There are too many variants to be researched, and it is very likely that many of the variants identified are not truly causal. The PLINK results from the combined datasets should be taken with caution.

Most of the genes and SNPs identified by PLINK in the combined analysis (Table 13) were not discussed earlier. The G5 domain containing protein is a hypothetical gene found in PA7. It is absent in all but 2 CF strains, but is present in 37/639 non-CF strains. G5 domains in proteins have been identified as N-acetylglucosamine binding sites, and could be related to biofilm formation, at least in *Streptococcus* [164]. Another hypothetical protein identified was a probable helicase. This gene has not been experimentally identified or studied, however some RNA helicases have been shown to impact biofilm formation [165, 166]. An A8S mutation in this gene was present in 15/947 CF strains, but only 1/639 non-CF strains. The E214D mutation was found almost evenly in CF and non-CF strains, appearing in 15 and 12 isolates respectively.

Table 13: Top genes and SNPs from from PLINK stratified association analysis

<i>Rank</i>	<i>Gene</i>	<i>Mutation</i>	<i>p value</i>
1	G5 domain containing protein		2.68×10^{-60}
2, 3	hypothetical helicase	A8S, E214D	1.59×10^{-49}
5	<i>fdhE</i>	A235T	1.79×10^{-45}
8	<i>glpD</i>	R274H	8.97×10^{-44}
10, 11	<i>metG</i>	V76A, E659Q	3.90×10^{-40}
12, 13	<i>pvdE</i>	E327S, S394E	5.12×10^{-40}
14	<i>tonB</i> dependent receptor	L14P	3.37×10^{-39}

Two mutations were identified in two proteins involved in metabolism. FdhE is involved with ferric iron binding and has been studied with regards to energy metabolism in bacteria [167], but has not been linked to CF-related adaptations. An A235T mutation was found in 28/947 CF strains and

Figure 27: QQ plot of calculated $\log p$ values in PLINK of the gene and SNP datasets against expected p values. The red line is when the observed p values equal the expected p values ($x=y$)

Figure 28: Manhattan plot of p values in the PLINK analysis of the gene and SNP dataset. Points above the red line are significant, and coloured in green. The red line is the genome-wide significance line, at $\log(1 \times 10^{-8})$. The blue line is the suggestive significance line, at $\log(1 \times 10^{-5})$.

47/639 non-CF PA strains. *glpD* encodes for a protein involved in glycerol metabolism. Mutations and changes its activity can affect the metabolism of amino acids, induce a stress response with *rpoS* [168], and lower alignate production making isolates much more virulent [169]. The R274H mutation was in only a single CF strain of PA and 38/639 non-CF strains.

MetG, or methionyl-tRNA ligase, is required to initiate and elongate protein synthesis. Changes in the C-terminus of the gene *metG* has been shown to cause increased virulence in *E. coli* [170], however the effects have not been studied in PA. The V76A mutation was only found in CF strains of PA (16/947, or 1.7%), while the E659Q mutation was found in both CF and non-CF isolates (215/947, or 22.7%, CF strains and 159/639, 24.9%, non-CF strains).

pvd genes are involved in the synthesis of pyoverdine, a siderophore. These genes are involved in iron acquisition [171], which is a virulence adaption of PA in a CF lung. Both the E327S and S394E mutations were in over half of the CF strains (537/947, 56.7%), but less than half of the non-CF strains (278/639 and 280/639 isolates, respectively, 43.5-43.8%). Most studies of the pyoverdine pathway and feedback loops are of *pvdS*, not *pvdE*, however this pathway does differ in CF environments compared non-CF ones [123, 22]. *pvdL* was found in a longitudinal PA study to have a high number of SNPs during chronic lung infection [17].

The importance of TonB receptors was discussed in earlier sections (Section 4.2), however the L14P mutation was not found in the SNP-only dataset analyses. This mutation was uncommon in the dataset, but found more often in non-CF strains (<1% of CF isolates, 7/947, but 3.4% of CF strains, 22/639).

6.2. treeWAS

Many of the genes identified in the treeWAS analysis of the combined gene and SNP dataset (Table 14, Figure 29) were already identified in previous PLINK and treeWAS sections (Sections 4.2, 4.3, 5.2 and 5.3). The one gene not previously discussed was *masA*, which encodes for enolase-phosphatase E-1, and was the only gene listed in Table 14 that was not previously discussed. The gene is more often found in non-CF strains of PA (608/639, but only 483/947 non-CF strains). A link between *masA* and adaptations to a CF lung environment has not been studied as of yet.

Figure 29: Manhattan plot of gene and SNP scores found from treeWAS association simultaneous analysis.

Table 14: Top genes and SNPs found in treeWAS simultaneous association analysis.

<i>Rank</i>	<i>Gene</i>	<i>Mutation</i>	<i>Association Score</i>
1	<i>pqqF</i>	H3R	50.5
2	<i>protease</i>		−50
3	<i>pqqF</i>	H12R	49.5
4	<i>hxcY</i>		−49
7	<i>napA</i>		−46
8	<i>masA</i>		−46
9	<i>tle1</i>	D468E	45
11	<i>iciA</i>	M1L	44
12	<i>tonB2</i>		−44
17	<i>recF</i>		−42
22	<i>cysH</i>	L2P	39

6.3. Random forest

As with the treeWAS combined analysis, many of the genes and SNPs in the random forest model (Table 15) were found and described in earlier sections (Sections 4.2, 4.4, 5.2 and 5.4).

Mutations within *mucD* and *argE* were not mentioned in earlier sections. MucD is a protein that regulates the alginate pathway by activating the degradation of MucA [172, 173]. Alginate production affects biofilm formation and virulence of bacterial strains. *mucD* does not play as large a role in mediating alginate as *mucA*, however finding a mutation in *mucD* that contributes to the adaptation of PA in a CF lung is not surprising. The V440I mutation was in 262/947 CF isolates (27.7%), 202/639 non-CF (31.6%). Acetylornithine deacetylase, encoded by *argE*, is involved in amino acid metabolism [174] and arginine biosynthesis [175]. Arginine synthesis has been predicted to be involved in biofilm formation [176], but this has not been experimentally confirmed.

Table 15: Top genes and SNPs that contribute to the random forest model.

<i>Rank</i>	<i>Gene</i>	<i>Mutation</i>	<i>Importance</i>
1	PA2074		0.262
2	<i>mucA</i>		0.073
3	<i>pqqF</i>	H3R	0.051
4	<i>tonB2</i>		0.038
9	<i>pqqF</i>	H12R	0.010
19	<i>wbpM</i>	V113P	0.005
23	<i>mucD</i>	V441I	0.005
24	<i>argE</i>	R221Q	0.005

6.4. Support vector machine

Several of the genes and SNPs in Table 16 were discussed previously (Sections 4.2, 5.4, and 5.5). The second and third genes identified with the SVM approach were probable dioxygenases, with no experimentally confirmed function. One is present in 448 of 947 CF and 309 of 639 non-CF strains, while the other is found in 497 of 947 CF and 319 of 639 non-CF isolates. Though the exact function of these genes is currently undescribed, various dioxygenases have been linked to virulence and prevalence of chronic PA infections [177]. Another poorly described protein identified was an isomerase, 2,3-dihydro-3-hydroxyanthranilate isomerase. This protein has not been experimentally studied, but is similar to a protein produced by *phzF*, which is part of the phenazine biosynthesis pathway. Another *phz* gene was previously discussed, *phzE*, in section 4.5. This isomerase was present in only 80 of 947 CF isolates, and 82 of 639 non-CF strains.

mexA encodes an efflux pump component, similar to *mexB*, *mexZ* and *mexK* previously discussed (Section 4.2). *mexA* was absent more often in CF isolates when compared with non-CF (absent in 53/947 CF, 5.6%, and 10/639 non-CF, 1.6%).

exaC produces an aldehyde dehydrogenase ExaC, which is part of energy and metabolism pathways [178]. Its role in growth in different environments is debated [178, 179], and has not been linked to beneficial or detrimental effects in CF lung environments. The gene was missing in more CF strains, present in only 52.7% of CF strains (499/947), but was present in 73.6% of non-CF strains (470/639).

Table 16: Top genes and SNPs found in support vector machine model

<i>Rank</i>	<i>Gene</i>	<i>Mutation</i>	<i>Absolute hyperplane coefficient</i>
1	<i>mucA</i>		0.0432
2, 3	dioxygenase		0.0337, 0.0320
4	isomerase		0.0315
5	<i>tonB2</i>		0.0300
7	<i>mexA</i>		0.0281
8	<i>yecS</i>	164 insertion	0.0279
9	<i>oprB</i>		0.0277
10	<i>exaC</i>		0.0262
16	<i>pirA</i>	A370T	0.0244
19	<i>mexZ</i>		0.0235

6.5. Conclusion

With the combined gene and SNP datasets, very few genes were found in common between the different analysis methods (Figure 30). No variants were in common between all four methods. Several variants identified in at least three of the methods were found in earlier analyses, such as *tonB2*, *mexZ*, *recF*, and the two mutations in *pqqF*.

PLINK did not have a lot of variants in common with the other methods, and identified over 2000 genes and SNPs that contributed to the CF-phenotype of PA. treeWAS, RF and SVM identified several variants in common, and ranked many of the top ones similarly (Figure 31). Between treeWAS, RF and SVM, none of the methods outperformed the others in terms of identifying genes/SNPs from literature.

Figure 30: Venn diagram of results from PLINK, treeWAS, random forest and support vector machine. Variants identified in at least 3 of the 4 methods are reported.

Figure 31: Heatmap of variants found in the combined gene and SNP datasets with various methods. Darker blue is a higher ranking (lower p value or higher association score/ importance/ hyperplane coefficient) variant. Yellow is a lower ranking variant. White is not identified as contributing to the phenotype.

7. SELECT GENE AND MUTATION DATASET RESULTS AND DISCUSSION

This dataset is comprised of the genes and mutations with an importance greater than 0 identified in the random forest model of the gene and mutation datasets (Sections 4.4 and 5.4). PLINK, treeWAS, RF and SVM were run on this smaller dataset, using the same settings as in previous analyses. Random forests are often utilized for feature selection in datasets with many features [180, 181]. I used the variants identified with the random forest model of previous datasets to select important genes and SNPs to be further analyzed by these various methods, in an attempt to gain more relevant and robust results, ideally corroborated by other analyses either with this dataset or previous ones.

Figure 32 is a heatmap of genes and SNPs identified in three of the four methods (PLINK, treeWAS, RF and SVM) with the reduced dataset. The darker the colour, the higher ranked the gene/SNP was in the analysis (ie. lower p value, higher absolute association score, importance or absolute hyperplane coefficient). This provides a comparison of genes and SNPs identified independently in multiple methods, and how highly the variant was ranked.

Many of the genes and SNPs found with PLINK and treeWAS were previously identified with the larger, original datasets. *mucA*, *hxcY*, and *pqqF* are examples of these. There was not a lot of overlap between SNPs and genes found with an SVM and the other methods. The random forest model and PLINK had a lot of overlap in their results, and RF ranked genes found with other methods quite highly. The two *pqqF* SNPs (H3R and H12R, found often in other methods) were ranked highly with all the methods in this dataset.

7.1. PLINK

Figure 33 shows the observed and expected p values using PLINK on the reduced dataset. The observed p values are much higher than expected, as found with other datasets. Observed p values may not follow a uniform distribution, as many non-causal variants are excluded in this dataset. The tail, or sharp increase, in observed p values is much less pronounced than in previous PLINK analyses.

The Manhattan plot (Figure 34) of the reduced dataset still does not show spikes of genes/SNPs inherited together. There are parts of the genome that were not important to CF adaptation, as there

Figure 32: Heatmap of genes and SNPs found in the reduced dataset with various methods. Dark blue is a higher ranking (lower p value or higher association score/ importance/ hyperplane coefficient) variant. Yellow is a lower ranking variant. White is not identified as contributing to the phenotype.

are much fewer points after position 5.5×10^6 .

7.2. treeWAS

Figure 35 is a Manhattan plot of the treeWAS simultaneous association scores for the reduced dataset. It is similar to the Manhattan plots of previous treeWAS analysis, with a few spikes of important genes/SNPs.

Figure 33: QQ plot of calculated $\log p$ values in PLINK of the reduced gene and SNP datasets against expected p values. The red line is when the observed p values equal the expected p values ($x=y$)

Figure 34: Manhattan plot of p values in the PLINK analysis of the reduced gene and SNP dataset. Points above the red line are significant, and coloured in green. The red line is the genome-wide significance line, at $\log(1 \times 10^{-8})$. The blue line is the suggestive significance line, at $\log(1 \times 10^{-5})$.

Figure 35: Manhattan plot of reduced gene and SNP scores found from treeWAS association simultaneous analysis.

8. RANDOMIZATIONS

Randomized analyses were run using the gene presence/absence dataset to evaluate the accuracy and results of the various methods. 1000 runs were performed, using the same settings as the original models, but randomly shuffling the phenotype, maintaining the same number of CF and non-CF strains. The randomized datasets act as an empirical null distribution, allowing us to infer the expected false positive rate for each method. That is, given a random set of data, how many associations does each method find?

8.1. PLINK

The PLINK analysis was run using the stratified case/control analysis and the populations as determined previously with fastStructure (Section 4). Figure 36 shows the distribution of p values over the 1000 runs. The blue line with colour under the curve is the distribution of p values from the run with the original dataset. The p values from randomized data trials were evenly distributed, while the original run had an excess of low p values. This suggests that the original dataset had valuable information pertaining to the CF phenotype of PA.

8.2. treeWAS

treeWAS was run using the randomized phenotypes and the original phylogeny from ExaML (Section 3.1). Figure 37 shows the distribution of p values in the original and randomized trials. The shaded blue curve is the distribution of p values with the original data. There is no obvious difference between the original p value distribution and the randomized ones, and thus results from treeWAS should be taken with caution. treeWAS creates a null dataset and determines the distribution of p values and associations from the null dataset. It then chooses a cutoff association score from this distribution, and assigns a p value of 0 to any variant in the actual dataset that is higher than the cutoff. It is unclear why the randomized data does not follow a uniform distribution that is distinct from the true data results.

Figure 36: Distribution of p-values from 1000 randomized PLINK model iterations. Blue, shaded line is the distribution of p-values from the original run.

Figure 37: Distribution of p-values from 1000 randomized treeWAS simultaneous association model iterations. Blue, shaded line is the distribution of p-values from the original run.

8.3. Random Forest and Support Vector Machine

With both machine learning methods, accuracy in the randomized datasets dropped from around 90% to 50%. Randomized RF trials had an accuracy of $52\% \pm 2.2\%$ (Figure 38), while SVM accuracy dropped to $51.8\% \pm 2.5\%$ (Figure 39). The RF models needed over 900 genes to explain the variance between CF and non-CF isolates, compared to 265 genes with the original model. This suggests that both the RF and SVM models were able to find meaningful genes within the dataset and that there were correlations between the phenotype and presence/absence of some genes.

8.4. Conclusion

In conclusion, PLINK, RF and SVM were not able to find meaningful differences in CF from non-CF strains when phenotype data was randomized. This is encouraging for the results from these methods, suggesting that true correlations were found between genotype and phenotype. treeWAS found correlations between genes/SNPs and the phenotype even with randomized data. The null hypothesis of having no correlation between genotype and phenotype has a similar distribution of p values as the model with the original dataset. Thus, we cannot reject the hypothesis that there is no correlation between the CF phenotype and some genes/SNPs using treeWAS.

9. CONCLUSION

Machine learning is an artificial intelligence method that has recently been used in human and bacterial GWAS. Both random forests and support vector machines have been used in GWAS with success, however large datasets (hundreds, ideally thousands, of samples) are required to provide meaningful results. Interestingly, these methods may produce less false positives when compared with non-machine learning statistical methods [106]. The use of machine learning in GWAS is expanding, and requires more study into which models are most appropriate for GWAS datasets. For example, one bacterial GWAS used PLINK, then a random forest, to find significant associations [89]. Two others used random forests alone, albeit with small sample sizes (42 and 13 genomes, respectively) [74, 95]. An SVM has been used in one study of 1595 strains of *M. tuberculosis*, and was successful in finding 33 known and 24 novel genes associated with antibiotic resistance [96].

Here, I used both machine learning and non-machine learning GWAS approaches to identify

Figure 38: Accuracy of 1000 randomized random forest model iterations. Red line is the accuracy of the model with original data.

Figure 39: Accuracy of 1000 randomized support vector machine model iterations. Red line is the accuracy of the model with original data.

CF-pathoadaptive variants in *P. aeruginosa*. *P. aeruginosa* is a ubiquitous bacterium that causes chronic lung infections in cystic fibrosis patients. Since these bacteria are directly related to the mortality and morbidity of CF patients, there is a large body of research on the treatment of these infections. PA has been shown to evolve in the lung to become better adapted to this unique environment. There have been several longitudinal studies examining changes in the bacterium over the course of a patient's lifespan and infection [17, 18, 19, 182]. Many of these changes during chronic infection include resistance to antibiotics, changes in expression of virulence factors, increases in mutation rate, formation of biofilms and changes in metabolism [14, 15]. I performed a GWAS to find novel evolutionary changes that PA undergoes in a CF lung, as well as differences between CF and non-CF populations of PA, and compared results between a variety of datasets and methods.

The full GWAS dataset consisted of 15,639 genes in the pan-genome, and 54,662 SNPs or indels in 4652 core genes. In the genome-wide association analyses, the separated gene and SNP datasets (1 and 2, Sections 4 and 5) seemed to perform the best. The fully combined dataset (dataset 3, Section 6) had no variants in common between PLINK, treeWAS, SVM and RF analyses. PLINK went from finding a few hundred variants, to finding a few thousand being significant to a CF-phenotype. TreeWAS stayed most consistent in results when combining datasets (Figure 40). SVM found many variants in common with the combined and separated datasets, however the hyperplane coefficients and ranks differed between the analyses. Random forest was most consistent between datasets 1, 2, and 3, although overall it also reported different variants between the different datasets.

With the gene presence/absence dataset (1, Section 4), a random forest gave the most promising performance, with many high ranking genes found in the literature, and some hypothetical genes for further research. An SVM was most promising with the SNP dataset (2, Section 5), with the fewest highly ranked hypothetical genes, and recovering more genes and SNPs from literature than the other methods.

The most important genes identified in the random forest analysis were involved in DNA repair (*recF*, *mutL*), antibiotic resistance (*mexF*), alginate production (*mucA*), metabolism/biosynthesis (*tonB2*), and signalling (*pdtA*). Several hypothetical proteins, including PA2074 and PA5037, were also identified in the top-ranking genes, and were recovered in other analyses. The importance values in the model decrease sharply after the first few genes. Further research should be concentrated on high ranking hypothetical proteins and other genes with unconfirmed function (such as PA1238, a

Figure 40: Variants in common between datasets for the various methods. A) PLINK, B) treeWAS, C) random forest, D) support vector machine. Datasets 1 and 2 (gene presence/absence and core SNPs) had no variants in common, as illustrated by blank spaces in the Venn diagrams.

probable outer membrane component of a multidrug efflux pump).

The SVM model identified some SNPs that are from known genes involved in DNA repair (*mutL*), antibiotic resistance (*mexK*, *pirA*, *gyrA*), host immune response evasion (*wbpM*), and host adaptation (*yecS*). Several mutations were found in a putative dipeptidase, PA2393, at positions 21 and 22 in the amino acid sequence. Several mutations identified by the SVM had low frequencies in the population; for example, V128A in *pirA* was only found in 29/947 CF isolates, and 9/631 non-CF. Some mutations found in hypothetical proteins include A137V in PA3923, V304A in probable major facilitator superfamily transporter PA2701, D172N in probable acyl-CoA dehydrogenase PA1631, A72R in PA3057, and S4F in probable ATP-dependent RNA helicase PA3466. These mutations were ranked 10-14 based on their absolute hyperplane coefficients. SVM was the only model to identify D87Y in *gyrA*, a mutation that has been found to increase drug resistance by altering the binding site of quinolones. The SVM ranked this mutation quite high (18/394), which gives us more confidence in the SVM results.

To validate these findings, simulations can be run with created datasets to observe reproducibility, confirm accuracy of models and their gene/SNP predictions. Experimentally, gene knockouts for top hypothetical proteins could be performed in the lab, to test the prediction that these genes influence CF-pathoadaptive phenotypes. Where genes are missing in lab strains, gene knock-ins could be performed to identify protein function and changes to the phenotype. Predictive modeling tools could be used prior to experimental tests to predict protein function and provide guidance as to possible phenotypic changes. For SNPs, competition assays could be run in various environments (including a pseudo-CF lung environment) to measure changes in fitness and potentially indicate drivers behind these evolutionary changes. Additionally, the genomes of PA isolated during longitudinal studies following a single patient could be tested to see how these genes/SNPs change over the course of an infection. To experimentally compare results between studies, hypothetical proteins in the top 10-15 results of each method could be determined through experimental gene knockouts. The results from these experiments could indicate which algorithms had the fewest false positives, and therefore the best results.

In this study, I have discovered that RF and SVM models were able to identify variants in genes that have been previously described in the literature as contributing to PA pathoadaptation, as well as hypothetical proteins that we can research further. Proteins involved in metabolism, alginate

pathways, antibiotic resistance and DNA repair were some of the highest ranked proteins in these methods. A number of the results from these methods overlapped with each other or PLINK. TreeWAS often recovered more hypothetical proteins than the other methods, in the most important genes. PLINK also identified a number of hypothetical proteins, but many of the top results were also found with RF and SVM models. PLINK likely had many false positive results, based on the QQ plots, identifying hundreds of genes and SNPs below the significance threshold.

In a broader sense, these results could be used in the future to aid in treatment of CF infections. For example, if some hypothetical proteins are characterized as contributing to antibiotic resistance, it could help inform physicians on which drug combination is best to combat the development of AMR. If there are proteins related to metabolism and biosynthesis pathways, there may be other treatment methods for the infections. Algorithms such as decision trees could provide diagnostic tools for PA infections, and help predict the spread of the bacterium in a population or within a patient. Machine learning, in general, could be used for more bacterial GWAS to find variants associated with a phenotype, even if the phenotype is rare. Some of these algorithms could also be used to see if there is a difference in genotype in differing severities of infection.

In conclusion, machine learning methods can provide insights into large genome datasets. Different models (RF, SVM or other algorithms) may be appropriate for various datasets, and multiple methods should be used to compare results and determine the most robust results. Several genes and mutations, both known and unknown, may contribute to CF lung adaption of *Pseudomonas aeruginosa*. These variants should be investigated further for their possible role in chronic PA infections.

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A. BACKGROUND INFORMATION

A.1. Genes/proteins associated with CF-phenotype of PA

Table 17: Genes and proteins important for the CF-phenotype as identified in several review papers.

<i>RefSeq</i>	<i>Gene name</i>	<i>Product name</i>
NP_251075.1	pvdQ	3-oxo-C12-homoserine lactone acylase PvdQ
NP_251852.1	rpsA	30S ribosomal protein S1
NP_253342.1	cupE6	Adhesin-like protein CupE6
NP_252771.1	cupB5	adhesive protein CupB5
NP_252235.1	algG	alginate-c5-mannuronan-epimerase AlgG
NP_253887.1	amgR	AmgR
NP_253886.1	amgS	AmgS
NP_253911.1	pepP	aminopeptidase P
NP_249454.1	mucA	anti-sigma factor MucA
NP_249649.1	oprD	outer membrane porin OprD precursor
NP_253212.1	ampD	beta-lactamase expression regulator AmpD
NP_252799.1	ampC	beta-lactamase precursor
NP_253057.1	bifA	BifA
NP_250819.1	cupA2	chaperone CupA2
NP_250822.1	cupA5	chaperone CupA5
NP_252774.1	cupB2	chaperone CupB2
NP_252772.1	cupB4	chaperone CupB4
NP_249684.1	cupC2	chaperone CupC2
NP_249104.1	chpA	component of chemotactic signal transduction system
NP_253389.1		conserved hypothetical protein
NP_253649.1		conserved hypothetical protein
NP_253820.1		conserved hypothetical protein
NP_253991.1	dadA	D-amino acid dehydrogenase, small subunit
NP_253703.1	aceF	dihydrolipoamide acetyltransferase
NP_251858.1	gyrA	DNA gyrase subunit A
NP_064724.1	gyrB	DNA gyrase subunit B
NP_253633.1	mutL	DNA mismatch repair protein MutL
NP_252310.1	mutS	DNA mismatch repair protein MutS
NP_252956.1	fusA1	elongation factor G
NP_250405.1	exsD	ExsD
NP_250818.1	cupA1	fimbrial subunit CupA1
NP_250821.1	cupA4	fimbrial subunit CupA4
NP_252770.1	cupB6	fimbrial subunit CupB6
NP_249683.1	cupC1	fimbrial subunit CupC1
NP_253341.1	cupE5	Fimbrial usher protein CupE5
NP_252907.1	phzS	flavin-containing monooxygenase
NP_253398.1	phuR	Heme/Hemoglobin uptake outer membrane receptor PhuR precursor
NP_252396.1	wspB	hypothetical protein
NP_252394.1	wspD	hypothetical protein
NP_251783.1		hypothetical protein
NP_253648.1		hypothetical protein

<i>RefSeq</i>	<i>Gene name</i>	<i>Product name</i>
NP_251103.1	pvdH	L-2,4-diaminobutyrate:2-ketoglutarate 4-aminotransferase, PvdH
NP_249004.1		L-cysteine transporter of ABC system YecS
NP_251076.1	pvdA	L-ornithine N5-oxygenase
NP_253885.1		LD-carboxypeptidase
NP_250509.1	cadA	lysine decarboxylase
NP_249118.1	oprM	multidrug efflux outer membrane protein OprM precursor
NP_250503.1	mltD	membrane-bound lytic murein transglycosylase D precursor
NP_249103.1	pilK	methyltransferase PilK
NP_251181.1	mexS	MexS
NP_250710.1	mexZ	MexZ
NP_253291.1	morA	motility regulator
NP_249115.1	mexR	multidrug resistance operon repressor MexR
NP_253846.1		multidrug resistance protein
NP_252264.1	nalD	NalD
NP_251543.1	oprI	Outer membrane lipoprotein OprI precursor
NP_251754.1	pelA	PelA
NP_253108.1	ftsI	penicillin-binding protein 3
NP_252901.1	phzC1	phenazine biosynthesis protein PhzC
NP_250592.1	phzC2	phenazine biosynthesis protein PhzC
NP_252902.1	phzD1	phenazine biosynthesis protein PhzD
NP_250593.1	phzD2	phenazine biosynthesis protein PhzD
NP_252903.1	phzE1	phenazine biosynthesis protein PhzE
NP_250594.1	phzE2	phenazine biosynthesis protein PhzE
NP_253340.1	cupE4	Pilin assembly chaperone CupE4
NP_253337.1	cupE1	Pilin subunit CupE1
NP_253338.1	cupE2	Pilin subunit CupE2
NP_253339.1	cupE3	Pilin subunit CupE3
NP_253464.1	pmrA	PmrA: two-component regulator system response regulator PmrA
NP_253747.1	phaF	polyhydroxyalkanoate synthesis protein PhaF
NP_248741.1	phzH	potential phenazine-modifying enzyme
NP_253492.1		POTRA-like domain-containing usher, CupB3
NP_252393.1	wspE	probable chemotaxis sensor/effector fusion protein
NP_252397.1	wspA	probable chemotaxis transducer
NP_253238.1		probable D-amino acid oxidase
NP_252775.1	cupB1	probable fimbrial subunit CupB1
NP_252392.1	wspF	probable methylesterase
NP_252899.1	phzA1	probable phenazine biosynthesis protein
NP_250590.1	phzA2	probable phenazine biosynthesis protein
NP_252900.1	phzB1	probable phenazine biosynthesis protein
NP_250591.1	phzB2	probable phenazine biosynthesis protein
NP_252904.1	phzF1	probable phenazine biosynthesis protein
NP_250595.1	phzF2	probable phenazine biosynthesis protein
NP_252898.1	phzM	probable phenazine-specific methyltransferase
NP_252395.1	wspC	probable protein methyltransferase
NP_252906.1	phzG1	probable pyridoxamine 5'-phosphate oxidase
NP_252905.1	phzG2	probable pyridoxamine 5'-phosphate oxidase

<i>RefSeq</i>	<i>Gene name</i>	<i>Product name</i>
NP_249226.1		probable transcriptional regulator
NP_250921.1	pslA	PslA
NP_251115.1	pvdG	PvdG
NP_251090.2	pvdJ	PvdJ
NP_251114.1	pvdL	PvdL
NP_251084.1	pvdN	PvdN
NP_251085.1	pvdO	PvdO
NP_251082.1	pvdP	PvdP
NP_251079.1	pvdR	PvdR
NP_251080.1	pvdT	PvdT
NP_251087.1	pvdE	pyoverdine biosynthesis protein PvdE
NP_251089.1	pvdD	pyoverdine synthetase D
NP_251086.1	pvdF	pyoverdine synthetase F
NP_253702.1	aceE	pyruvate dehydrogenase
NP_250589.1	qscR	quorum-sensing control repressor
NP_249552.1	rbdA	RbDA
NP_249116.1	mexA	multidrug efflux membrane fusion protein MexA precursor
NP_249117.1	mexB	multidrug efflux transporter MexB
NP_253543.1	retS	RetS (Regulator of Exopolysaccharide and Type III Secretion)
NP_253152.1	rpoN	RNA polymerase sigma-54 factor
NP_249619.1	gacS	sensor/response regulator hybrid
NP_249749.1	shaE	ShaE
NP_249453.1	algU	sigma factor AlgU
NP_251116.1	pvdS	sigma factor PvdS
NP_253654.1	parE	topoisomerase IV subunit B
NP_252798.1	ampR	transcriptional regulator AmpR
NP_250121.1	lasR	transcriptional regulator LasR
NP_251182.1	mexT	transcriptional regulator MexT
NP_253290.1	nfxB	transcriptional regulator NfxB
NP_249099.1	pilG	twitching motility protein PilG
NP_249100.1	pilH	twitching motility protein PilH
NP_249101.1	pilI	twitching motility protein PilI
NP_249102.1	pilJ	twitching motility protein PilJ
NP_249086.1	pilT	twitching motility protein PilT
NP_249087.1	pilU	twitching motility protein PilU
NP_253237.1	pilR	two-component response regulator PilR
NP_253236.1	pilS	two-component sensor PilS
NP_253727.1	pilQ	type 4 fimbrial biogenesis protein PilQ precursor
NP_253216.1	pilB	type 4 fimbrial biogenesis protein PilB
NP_253246.1	pilE	type 4 fimbrial biogenesis protein PilE
NP_252494.1	pilF	type 4 fimbrial biogenesis protein PilF
NP_253731.1	pilM	type 4 fimbrial biogenesis protein PilM
NP_253730.1	pilN	type 4 fimbrial biogenesis protein PilN
NP_253729.1	pilO	type 4 fimbrial biogenesis protein PilO
NP_253728.1	pilP	type 4 fimbrial biogenesis protein PilP
NP_253241.1	pilV	type 4 fimbrial biogenesis protein PilV
NP_253242.1	pilW	type 4 fimbrial biogenesis protein PilW

<i>RefSeq</i>	<i>Gene name</i>	<i>Product name</i>
NP_253243.1	pilX	type 4 fimbrial biogenesis protein PilX
NP_253244.1	pilY1	type 4 fimbrial biogenesis protein PilY1
NP_253245.1	pilY2	type 4 fimbrial biogenesis protein PilY2
NP_251650.1	pilZ	type 4 fimbrial biogenesis protein PilZ
NP_253215.1	pilA	type 4 fimbrial precursor PilA
NP_253218.1	pilD	type 4 prepilin peptidase PilD
NP_253910.1	ubiH	ubiH protein
NP_252709.1	mpl	UDP-N-acetylmuramate: L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase
NP_250820.1	cupA3	usher CupA3
NP_252773.1	cupB3	usher CupB3
NP_249685.1	cupC3	usher CupC3
NP_248781.1	vgrG1	VgrG1
NP_250202.1	vgrG2a	VgrG2a
NP_248953.1	vgrG2b	VgrG2b
NP_251063.1	vgrG3	VgrG3
NP_251375.1	vgrG4	VgrG4
NP_251984.1	vgrG4a	VgrG4a
NP_252176.1	vgrG4b	VgrG4b
NP_253777.1	vgrG5	VgrG5
NP_253953.1	vgrG6	VgrG6
NP_252391.1	wspR	WspR
	mutL	DNA mismatch repair protein MutL (pseudogene)
	mutS	DNA mismatch repair protein MutS (pseudogene)

B. RESULTS

B.1. Dataset 1 PLINK results

Table 18: Genes identified with PLINK from dataset 1, gene presence/absence. Genes with p values less than or equal to 2.54×10^{-10} are reported.

Rank	Accession	Gene name	Protein name	p value	Odds ratio
1	NP_248888.1	tonB2	TonB2	3.09×10^{-33}	41.62
2			non-ribosomal peptide synthetase (pseudogene)	1.32×10^{-28}	27.22
3	NP_251800.1	recF	ffs	1.70×10^{-26}	31.17
4			PA3110	4.36×10^{-25}	15.85
5	NP_064723.1		recF	1.66×10^{-20}	125.1
6			DNA-binding response regulator (pseudogene)	2.48×10^{-20}	2077
7	NP_249603.1		nan	3.14×10^{-20}	22.75
8			PA0912	8.84×10^{-20}	712.5
9	NP_253159.1		nan	1.07×10^{-19}	9.60
10			PA4469	2.25×10^{-19}	NA
11	NP_253319.1		PA4629	6.21×10^{-19}	NA

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>p value</i>	<i>Odds ratio</i>
12	NP_249620.1		two-component response regulator	1.63×10^{-18}	20.04
13	NP_253284.1		PA4594 probable ATP-binding component of ABC transporter	2.13×10^{-18}	2023
14	NP_250230.1		PA1539	2.39×10^{-18}	486.5
15			nan	2.89×10^{-18}	2093
16	NP_249381.1	<i>pdtA</i>	phosphate depletion regulated TPS partner A, PdtA	4.08×10^{-18}	22.15
17	NP_249374.1	<i>hxcY</i>	HxcY	6.46×10^{-18}	37.15
18	NP_249929.1		PA1238 probable outer membrane component of multidrug efflux pump	6.70×10^{-18}	24.35
19	NP_250420.1		PA1729	1.33×10^{-17}	501.3
20	NP_249865.1	<i>napA</i>	periplasmic nitrate reductase protein NapA	2.62×10^{-17}	28.32
21			hypothetical protein (pseudogene)	3.34×10^{-17}	171.8
22	NP_250018.1		PA1327 probable protease	5.99×10^{-17}	253.1
23			ABC transporter ATP-binding protein (pseudogene)	6.07×10^{-17}	NA
24	NP_250710.1	<i>mexZ</i>	MexZ	6.61×10^{-16}	9.85
25	NP_251876.1	<i>oprB</i>	Glucose/carbohydrate outer membrane porin OprB precursor	1.38×10^{-15}	6.32
26			nan	1.86×10^{-15}	31.26
27	NP_253331.1		PA4642	2.70×10^{-15}	14.69
28			nan	5.63×10^{-15}	27.87
29			sulfonate ABC transporter substrate-binding protein (pseudogene)	5.72×10^{-15}	21.64
30	NP_249454.1	<i>mucA</i>	mucA	5.73×10^{-15}	23.65
31			mutL (pseudogene)	7.61×10^{-15}	20.65
32			precorrin-3B C(17)-methyltransferase (pseudogene)	2.76×10^{-14}	22.18
33	NP_249117.1	<i>mexB</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux transporter MexB	3.61×10^{-14}	18.67
34			cysteine dioxygenase (pseudogene)	6.98×10^{-14}	20.06
35	NP_253474.1		PA4786 probable short-chain dehydrogenase	7.23×10^{-14}	28.39
36	NP_250376.1	<i>masA</i>	enolase-phosphatase E-1	7.61×10^{-14}	31.37
37	NP_253724.1		PA5037	1.05×10^{-13}	33.52
38			3-oxoacyl-ACP reductase (pseudogene)	3.30×10^{-13}	22.04
39	NP_249234.1		PA0543	4.70×10^{-13}	1438

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>p value</i>	<i>Odds ratio</i>
40			hemagglutinin (pseudogene)	5.24×10^{-13}	26.58
41	NP_253664.1	<i>aruI</i>	2-ketoarginine decarboxylase, AruI	5.86×10^{-13}	25.00
42			aldehyde dehydrogenase (pseudogene)	1.18×10^{-12}	NA
43	NP_251618.1		PA2928	1.28×10^{-12}	13.76
44	NP_251285.1		PA2595	1.30×10^{-12}	8.36
45	NP_253633.1	<i>mutL</i>	DNA mismatch repair protein MutL	1.63×10^{-12}	15.24
46			DeoR family transcriptional regulator (pseudogene)	2.45×10^{-12}	NA
47	NP_252760.1		PA4071	3.68×10^{-12}	11.86
48			phosphoenolpyruvate carboxylase (pseudogene)	4.42×10^{-12}	57.97
49			helix-turn-helix transcriptional regulator (pseudogene)	4.66×10^{-12}	58.68
50			TetR family transcriptional regulator (pseudogene)	5.34×10^{-12}	11.32
51	NP_251292.1		3-mercaptopropionate dioxygenase	5.51×10^{-12}	9.07
52	NP_250800.1		PA2110	5.80×10^{-12}	7.69
53	NP_252377.1	<i>ppc</i>	phosphoenolpyruvate carboxylase	6.17×10^{-12}	NA
54	NP_250181.1		PA1490 probable transcriptional regulator	6.59×10^{-12}	79.04
55			nan	6.84×10^{-12}	1217
56			hypothetical protein (pseudogene)	8.86×10^{-12}	115.6
57	NP_248954.1	<i>hcpC</i>	secreted protein Hcp	9.51×10^{-12}	0.21
58	NP_253650.1		PA4963	9.65×10^{-12}	106.3
59			nan	9.85×10^{-12}	11.16
60			high-affinity branched-chain amino acid ABC transporter ATP-binding protein LivG (pseudogene)	1.00×10^{-11}	NA
61			MFS transporter (pseudogene)	1.19×10^{-11}	24.73
62	NP_252036.1	<i>hsbR</i>	HptB-dependent secretion and biofilm regulator HsbR	1.20×10^{-11}	34.36
63	NP_252019.1		PA3329	1.95×10^{-11}	63.99
64			hypothetical protein (pseudogene)	2.12×10^{-11}	NA
65			hypothetical protein (pseudogene)	2.18×10^{-11}	11.21
66			[]	2.38×10^{-11}	5.92
67			pyridoxamine 5'-phosphate oxidase (pseudogene)	3.70×10^{-11}	15.59

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>p value</i>	<i>Odds ratio</i>
68			polyhydroxybutyrate depolymerase (pseudogene)	3.72×10^{-11}	1231
69			cell division protein (pseudogene)	4.02×10^{-11}	17.61
70	NP_252240.1	<i>algF</i>	alginate o-acetyltransferase AlgF	4.46×10^{-11}	21.32
71			nan	6.46×10^{-11}	14.2
72			alginate O-acetyltransferase (pseudogene)	7.28×10^{-11}	34.78
73			[]	7.75×10^{-11}	NA
74			nan	8.37×10^{-11}	NA
75	NP_249718.1	<i>amaB</i>	delta1-Piperidine-6-carboxylate dehydrogenase	8.41×10^{-11}	15.9
76			nan	8.70×10^{-11}	4.78
77	NP_249943.1	<i>dpkA</i>	DpkA	9.23×10^{-11}	20.55
78	NP_251108.1		PA2418	1.13×10^{-10}	18.09
79	NP_249762.1	<i>braF</i>	branched-chain amino acid transport protein BraF	1.36×10^{-10}	59.39
80			fused response regulator/phosphatase (pseudogene)	1.37×10^{-10}	58.28
81	NP_250038.1		PA1347 probable transcriptional regulator	1.47×10^{-10}	21.64
82			transporter (pseudogene)	1.64×10^{-10}	1082
83	NP_253532.1	<i>dipZ</i>	thiol:disulfide interchange protein DipZ	1.66×10^{-10}	52.20
84			amino acid ABC transporter permease (pseudogene)	1.74×10^{-10}	53.29
85	NP_253820.1		PA5133	1.91×10^{-10}	10.41
86			hypothetical protein	2.54×10^{-10}	NA
87			hypothetical protein	2.54×10^{-10}	NA
88			hypothetical protein	2.54×10^{-10}	NA
89			DNA cytosine methyltransferase (pseudogene)	2.54×10^{-10}	NA
90			nan	2.54×10^{-10}	NA
91			hypothetical protein	2.54×10^{-10}	NA
92			NAD-dependent deacylase (pseudogene)	2.54×10^{-10}	NA
93			integrase	2.54×10^{-10}	NA
94			integrase	2.54×10^{-10}	NA
95			hypothetical protein	2.54×10^{-10}	NA
96			general secretion pathway protein GspH (pseudogene)	2.54×10^{-10}	NA
97			hypothetical protein	2.54×10^{-10}	NA
98			hypothetical protein	2.54×10^{-10}	NA
99			hypothetical protein	2.54×10^{-10}	NA

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>p value</i>	<i>Odds ratio</i>
100			hypothetical protein	2.54×10^{-10}	NA
101			hypothetical protein	2.54×10^{-10}	NA
102			hypothetical protein	2.54×10^{-10}	NA
103			transcriptional regulator (pseudogene)	2.54×10^{-10}	NA
104	NP_249272.1		PA0581	2.54×10^{-10}	0
105	NP_249555.1		PA0864 probable transcriptional regulator	2.54×10^{-10}	0
106	NP_249735.1		PA1044	2.54×10^{-10}	0
107	NP_250357.1	<i>lip2</i>	Lip2	2.54×10^{-10}	0
108	NP_251440.1		PA2750	2.54×10^{-10}	0
109	NP_252529.1		PA3840	2.54×10^{-10}	0

B.2. Dataset 1 treeWAS results

Table 19: Genes identified with treeWAS from dataset 1, gene presence/absence.

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Association score</i>	<i>p value</i>
1	NP_250018.1		PA1327 probable protease	-50	0
2	NP_249374.1	<i>hxcY</i>	HxcY	-49	0
3	NP_250230.1		PA1539	-49	0
4	NP_250420.1		PA1729	-48	0
5	NP_249865.1	<i>napA</i>	periplasmic nitrate reductase protein NapA	-46	0
6	NP_250376.1	<i>masA</i>	enolase-phosphatase E-1	-46	0
7	NP_248888.1	<i>tonB2</i>	tonB2	-45	0
8	NP_251618.1		PA2928	-45	0
9	NP_064723.1	<i>recF</i>	recF	-43	0
10			nan	42	0
11	NP_249603.1		PA0912	-40	0
12			hypothetical protein	37	0
13	NP_250764.1		PA2074	37	0
14	NP_251800.1		PA3110	-37	0
15	NP_253724.1		PA5037	-35	0
16	NP_251981.1	<i>tli1</i>	Tli1	34	0
17			[]	33	0
18			putative rubredoxin reductase	33	0
19			hypothetical protein	30	0
20			nan	30	0
21			ABC transporter substrate-binding protein	29	0
22			mammalian cell entry protein (pseudogene)	28	0
23			hypothetical protein (pseudogene)	28	0
24	NP_248731.1		PA0041 probable hemagglutinin	-28	0

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Association score</i>	<i>p value</i>
25			hypothetical protein	27	0
26			hypothetical protein	27	0
27			glyA	26	0
28			hypothetical protein	26	0
29	NP_250105.1		PA1414	26	0
30	NP_250632.1		PA1942	26	0
31			hypothetical protein	25	0
32			cytidine deaminase	25	0
33			hypothetical protein	25	0
34			hypothetical protein	25	0
35			DNA repair protein	25	0
36			glycosyl hydrolase	25	0
37			hypothetical protein (pseudogene)	24	0
38			hypothetical protein (pseudogene)	24	0
39	NP_248954.1	<i>hcpC</i>	secreted protein Hcp	-24	0
40	NP_251089.1	<i>pvdD</i>	pyoverdine synthetase D	-24	0
41	NP_251148.1		PA2458	-24	0
42	NP_251175.1		PA2485	24	0
43	YP_003933614.1	<i>fdnG</i>	formate dehydrogenase-O, major subunit	-24	0
44			hypothetical protein	23	0
45			TetR family transcriptional regulator (pseudogene)	23	0
46			hypothetical protein	23	0
47			hypothetical protein	23	0
48			hypothetical protein	23	0
49			hypothetical protein	23	0
50			hypothetical protein	23	0
51			hypothetical protein	23	0
52			hypothetical protein	23	0
53			hypothetical protein	23	0
54			ffs	23	0
55			hypothetical protein	23	0
56			cytochrome C oxidase subunit II	23	0
57			universal stress protein	23	0
58			hypothetical protein	23	0
59			nitrate reductase catalytic subunit (pseudogene)	22	0
60			[]	22	0
61			transcriptional regulator (pseudogene)	22	0
62			hypothetical protein	-22	0
63			hypothetical protein	22	0
64			hypothetical protein	22	0
65			hypothetical protein	-22	0
66			hypothetical protein	22	0

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Association score</i>	<i>p value</i>
67			phosphoenolpyruvate synthase	22	0
68			hypothetical protein	22	0
69			hypothetical protein	22	0
70			hypothetical protein	22	0
71			hypothetical protein	22	0
72			hypothetical protein	22	0
73			ferredoxin (pseudogene)	22	0
74			monoamine oxidase	22	0
75			hypothetical protein	22	0
76	NP_250710.1	<i>mexZ</i>	MexZ	-22	0
77			aminotransferase	21	0
78			recombinase XerC	21	0
79			nan	21	0
80			aminopeptidase (pseudogene)	21	0
81			hypothetical protein	21	0
82			hypothetical protein	21	0
83			hypothetical protein	21	0
84			hypothetical protein	21	0
85			hypothetical protein	21	0
86			hypothetical protein	21	0
87			hypothetical protein	21	0
88			hypothetical protein	21	0
89			hypothetical protein (pseudogene)	21	0
90			hypothetical protein	-21	0
91			hypothetical protein	21	0
92			hypothetical protein	21	0
93			transporter	21	0
94			hypothetical protein	21	0
95			hypothetical protein (pseudogene)	21	0
96			ATP-binding protein	21	0
97	NP_250674.1	<i>exaC</i>	NAD+ dependent aldehyde dehydrogenase ExaC	-21	0
98	NP_253378.1		PA4689	-21	0
99			3-oxoacyl-ACP synthase	20	0
100			hypothetical protein	20	0
101			non-ribosomal peptide synthetase (pseudogene)	20	0
102			hypothetical protein	-20	0
103			flagellar export chaperone FliS	20	0
104			protease (pseudogene)	20	0
105			hypothetical protein	20	0
106			hypothetical protein	20	0
107			response regulator GacA (pseudogene)	20	0
108			hypothetical protein	20	0
109			hypothetical protein	20	0

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Association score</i>	<i>p value</i>
110			hypothetical protein	20	0
111			acyl carrier protein	20	0
112			methionine aminopeptidase	-20	0
113			methionine aminopeptidase	20	0
114			hypothetical protein	20	0
115			ffs	20	0
116			hypothetical protein (pseudogene)	20	0
117			hypothetical protein	20	0
118			hypothetical protein (pseudogene)	20	0
119			hypothetical protein	20	0
120			hypothetical protein	20	0
121			hypothetical protein	20	0
122			beta-lactamase	-20	0
123			hypothetical protein	20	0
124			nan	20	0
125			nan	20	0
126			[]	20	0
127	NP_249785.1	<i>fliD</i>	flagellar capping protein FliD	-20	0
128	NP_250876		PA2186	-20	0
129	NP_251152.1		PA2462	-20	0

B.3. Dataset 1 random forest results

Table 20: Genes identified with RF from dataset 1, gene presence/absence. Genes with importances greater than or equal to 0.002 are reported.

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Importance</i>
1	NP_250764.1		PA2074	0.237
2	NP_249454.1	<i>mucA</i>	anti-sigma factor MucA	0.123
3	NP_248888.1	<i>tonB2</i>	TonB2	0.084
4	NP_253724.1		PA5037	0.041
5	NP_064723.1	<i>recF</i>	RecF protein	0.029
6	NP_252532		PA3843	0.022
7			non-ribosomal peptide synthetase (pseudogene)	0.015
8	NP_251184.1	<i>mexF</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux transporter MexF	0.011
9			DNA-binding response regulator (pseudogene)	0.01
10	NP_249929.1		PA1238 probable outer membrane component of multidrug efflux pump	0.009
11			mutL (pseudogene)	0.009
12	NP_249381.1	<i>pdtA</i>	phosphate depletion regulated TPS partner A, PdtA	0.008

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Importance</i>
13	NP_253820.1		PA5133	0.008
14	NP_248948.1		PA0257	0.006
15	NP_250118		PA1427	0.006
16	NP_251134.1	<i>glyA2</i>	serine hydroxymethyltransferase	0.005
17	NP_249415.1		PA0724 probable coat protein A of bacteriophage Pf1	0.005
18	NP_250074.1		PA1383	0.005
19			hypothetical protein	0.005
20	NP_252177.1	<i>tle5</i>	Tle5	0.005
21			TetR family transcriptional regulator (pseudogene)	0.005
22	NP_249374.1	<i>hxcY</i>	HxcY	0.004
23	NP_249842.1	<i>imm2</i>	pyocin S2 immunity protein	0.004
24			nan	0.004
25	NP_250018.1		PA1327 probable protease	0.004
26	NP_253474.1		PA4786 probable short-chain dehydrogenase	0.004
27			nan	0.004
28	NP_250030.1	<i>aatP</i>	AatP	0.004
29	NP_249843		PA1152	0.004
30	NP_248851		PA0161	0.004
31	NP_250980.1	<i>gcd</i>	glucose dehydrogenase	0.004
32	NP_253775.1		PA5088	0.003
33			hypothetical protein	0.003
34	NP_253847.1		drug efflux transporter	0.003
35			transcriptional regulator	0.003
36	NP_249865.1	<i>napA</i>	periplasmic nitrate reductase protein NapA	0.003
37			peptidase	0.003
38	NP_249683.1	<i>cupC1</i>	fimbrial subunit CupC1	0.003
39			cystine ABC transporter substrate-binding protein (pseudogene)	0.003
40	NP_250246.1	<i>ccoP2</i>	Cytochrome c oxidase, cbb3-type, CcoP subunit	0.003
41	NP_253337.1	<i>cupE1</i>	Pilin subunit CupE1	0.003
42	NP_253159.1		PA4469	0.003
43	NP_249234.1		PA0543	0.003
44	NP_249116.1	<i>mexA</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux membrane fusion protein MexA precursor	0.003
45			hypothetical protein	0.003
46	NP_252799.1	<i>ampC</i>	beta-lactamase precursor	0.003
47	NP_248963.1		PA0272 probable transcriptional regulator	0.003
48			hypothetical protein (pseudogene)	0.003
49			DNA-binding protein	0.003
50			16S ribosomal RNA	0.003

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Importance</i>
51			nan	0.002
52	NP_249117.1	<i>mexB</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux transporter MexB	0.002
53	NP_251838.1	<i>wbpI</i>	UDP-N-acetylglucosamine 2-epimerase WbpI	0.002
54	NP_253664.1	<i>aruI</i>	2-ketoarginine decarboxylase, AruI	0.002
55			hypothetical protein	0.002
56	NP_250405.1	<i>exsD</i>	ExsD	0.002
57			hypothetical protein	0.002
58	NP_249669.1		PA0978	0.002
59			YD repeat (two copies)	0.002
60			3-oxoacyl-ACP reductase (pseudogene)	0.002
61	NP_250876		PA2186	0.002
62	NP_252773.1	<i>cupB3</i>	usher CupB3	0.002
63	NP_250073.1		PA1382 probable type II secretion system protein	0.002
64			hypothetical protein	0.002
65			hypothetical protein	0.002
66	NP_250871.1		PA2181	0.002
67			hypothetical protein	0.002
68			nan	0.002
69			hypothetical protein (pseudogene)	0.002
70	NP_250866.1		PA2176	0.002
71			nan	0.002
72			hypothetical protein	0.002
73	NP_252709.1	<i>mpl</i>	UDP-N-acetylmuramate:L-alanyl- gamma-D-glutamyl-meso- diaminopimelate ligase	0.002
74	NP_250059.1		PA1368	0.002
75			tryptophan repressor binding protein	0.002
76			transporter	0.002
77			UDP-N-acetylmuramate:L-alanyl- gamma-D-glutamyl- meso-diaminopimelate ligase (pseudogene)	0.002
78	NP_250796.1		PA2106	0.002
79			efflux transporter periplasmic adaptor subunit (pseudogene)	0.002
80	NP_250287.1	<i>htpG</i>	heat shock protein HtpG	0.002
81			hypothetical protein	0.002
82	NP_249339.1		PA0648	0.002
83			mercuric transporter periplasmic component	0.002
84	NP_252674.1		PA3985	0.002
85	NP_250817.1	<i>cgrA</i>	cupA gene regulator A, CgrA	0.002

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Importance</i>
86			hypothetical protein	0.002
87			nan	0.002
88			hypothetical protein	0.002
89			fused response regulator/phosphatase (pseudogene)	0.002

B.4. Dataset 1 support vector machine results

Table 21: Genes identified with SVM from dataset 1, gene presence/absence. Top 100 genes with absolute hyperplane coefficients greater than 0.03 are reported.

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Importance</i>
1	NP_249454.1	<i>mucA</i>	anti-sigma factor MucA	-0.0896
2	NP_251876.1	<i>oprB</i>	Glucose/carbohydrate outer membrane porin OprB precursor	-0.0875
3	NP_250710.1	<i>mexZ</i>	MexZ	-0.0727
4	NP_252903.1	<i>phzE1</i>	phenazine biosynthesis protein PhzE	-0.0654
5	NP_251417		PA2727	-0.0619
6	NP_248888.1	<i>tonB2</i>	TonB2	-0.0584
7			nan	0.0574
8			nan	-0.0535
9	NP_251836.1	<i>wbpK</i>	probable NAD-dependent epimerase/dehydratase WbpK	-0.0523
10	NP_253378.1		PA4689	-0.0495
11			hypothetical protein	0.0488
12			hypothetical protein	0.0487
13	NP_251134.1	<i>glyA2</i>	serine hydroxymethyltransferase	-0.0484
14			tRNA-Ile	-0.0484
15			RNA polymerase subunit sigma (pseudogene)	0.0484
16			aldehyde dehydrogenase (pseudogene)	0.0470
17			elongation factor Tu	0.0468
18			transcriptional regulator	0.0453
19	NP_252906.1	<i>phzG1</i>	probable pyridoxamine 5'-phosphate oxidase	-0.0448
20			tRNA-Ala	-0.0447
21	NP_249374.1	<i>hxcY</i>	HxcY	-0.0443
22	NP_249336.1		PA0645	0.0441
23	NP_251148.1		PA2458	-0.0441
24	NP_251147.1		PA2457	-0.0440
25			hypothetical protein (pseudogene)	-0.0431
26			non-ribosomal peptide synthetase (pseudogene)	-0.0430
27			nan	-0.0428
28	NP_250922.1	<i>pslB</i>	PslB	-0.0427

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Importance</i>
29	NP_249416.1		PA0725 hypothetical protein of bacteriophage Pf1	-0.0426
30	NP_251800.1		PA3110	-0.0426
31			TetR family transcriptional regulator (pseudogene)	0.0424
32	NP_249413.1		PA0722 hypothetical protein of bacteriophage Pf1	-0.0415
33	NP_249409.1		PA0718 hypothetical protein of bacteriophage Pf1	-0.0413
34	NP_251756.1		PA3066	-0.04052
35	NP_251757.1		PA3067 probable transcriptional regulator	-0.0405
36			nan	0.0404
37			hypothetical protein	0.0398
38			mutL (pseudogene)	0.0396
39			hypothetical protein	-0.0395
40			nan	-0.0394
41	NP_250914.1		PA2224	-0.0393
42	NP_249414.1	<i>coaB</i>	coat protein B of bacteriophage Pf1	-0.0391
43	NP_252888.1		PA4199 probable acyl-CoA dehydrogenase	-0.0390
44			nan	0.0385
45			mammalian cell entry protein (pseudogene)	0.0385
46			nan	0.0383
47	NP_253773		PA5086	0.0381
48	YP_003933611.1ccoQ2		Cytochrome c oxidase, cbb3-type, CcoQ subunit	0.0377
49			tRNA-Gly	-0.0377
50			hypothetical protein	0.0376
51	NP_253724.1		PA5037	-0.0376
52			hypothetical protein	-0.0373
53	NP_251122.1	<i>bexR</i>	bistable expression regulator, BexR	-0.0372
54	NP_249841.1	<i>pys2</i>	pyocin S2	-0.0371
55			nan	0.0367
56	NP_251418		PA2728	0.0366
57	NP_251755.1		PA3065	-0.0364
58			hypothetical protein	-0.0364
59			hypothetical protein	-0.0362
60	NP_249408		PA0717 hypothetical protein of bacteriophage Pf1	-0.0359
61			hypothetical protein	0.0355
62			hypothetical protein	0.0354
63	NP_249252.1		PA0561	0.0354
64			N-acetyltransferase GCN5	0.0352
65			nan	-0.0351
66			hypothetical protein	0.0350
67			16S ribosomal RNA	-0.0347
68	NP_252902.1	<i>phzD1</i>	phenazine biosynthesis protein PhzD	-0.0346

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Importance</i>
69			hypothetical protein	0.0346
70	NP_252711.1	<i>hdhA</i>	hydrazine dehydrogenase, HdhA	-0.0345
71	NP_253775.1		PA5088	-0.0345
72	NP_252900.1	<i>phzB1</i>	probable phenazine biosynthesis protein	0.0344
73	NP_253000.1	<i>pctB</i>	chemotactic transducer PctB	0.0341
74			group II intron reverse transcriptase/maturase	0.0338
75	NP_250632.1		PA1942	0.0337
76			tRNA-Arg	-0.0337
77	NP_251835.1	<i>wbpL</i>	glycosyltransferase WbpL	0.0335
78			[]	0.0333
79			hypothetical protein	-0.0332
80			hypothetical protein	-0.0332
81	NP_249381.1	<i>pdtA</i>	phosphate depletion regulated TPS partner A, PdtA	-0.0331
82			Rhs element Vgr protein (pseudogene)	-0.0331
83	NP_249116.1	<i>mexA</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux membrane fusion protein MexA precursor	-0.0330
84			hypothetical protein	0.0327
85			nan	0.0325
86			hypothetical protein	-0.0325
87	NP_253954.1	<i>hcpB</i>	secreted protein Hcp	-0.0324
88			5S ribosomal RNA	0.0324
89			integrase	0.0324
90	NP_251020.1		PA2330	-0.0323
91			UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase (pseudogene)	0.0322
92	NP_249117.1	<i>mexB</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux transporter MexB	-0.0322
93			hypothetical protein	0.0322
94			nan	0.0322
95			nucleotide pyrophosphohydrolase	-0.0321
96			phenazine biosynthesis protein phzD	0.0321
97			hypothetical protein (pseudogene)	-0.0320
98			hypothetical protein	-0.0320
99			nan	-0.0318
100			paraquat-inducible protein A	0.0318

B.5. Dataset 2 PLINK results

Table 22: SNPs identified with PLINK from dataset 2, SNPs within core genes. Top 100 SNPs with p values less than 1×10^{-8} are reported.

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>p value</i>	<i>Odds ratio</i>
1	NP_253781.1	<i>pqqF</i>	PA5094 probable ATP-binding component of ABC transporter	269 deletion	3.321×10^{-27}	0.78
2	NP_253781.1		PA5094 probable ATP-binding component of ABC transporter	X277Q	3.321×10^{-27}	0.78
3	NP_253957.1		PA5270	S5A	2.598×10^{-25}	9.21
4	NP_253462.1		PA4774	308 deletion	1.26×10^{-22}	0.10
5	NP_253462.1		PA4774	X350E	1.26×10^{-22}	0.10
6	NP_252321.1		PA3631	377 deletion	1.866×10^{-22}	0.10
7	NP_252321.1		PA3631	X409L	1.866×10^{-22}	0.10
8	NP_253373.1		PA4684	407 deletion	3.157×10^{-21}	0.11
9	NP_253373.1		PA4684	X433L	3.157×10^{-21}	0.11
10	NP_250663.1		pyrroloquinoline quinone biosynthesis protein F	H12R	4.306×10^{-21}	0.14
11	NP_253159.1	<i>proA</i>	PA4469	P137S	2.914×10^{-19}	NA
12	NP_253284.1		PA4594 probable ATP-binding component of ABC transporter	A127G	1.146×10^{-18}	2017
13	NP_253284.1		PA4594 probable ATP-binding component of ABC transporter	T213A	2.145×10^{-18}	2009
14	NP_253284.1		PA4594 probable ATP-binding component of ABC transporter	A226T	2.514×10^{-18}	2004
15	NP_253284.1		PA4594 probable ATP-binding component of ABC transporter	L101M	2.832×10^{-18}	2003
16	NP_250036.1	<i>pqqF</i>	PA1345	L17P	4.235×10^{-18}	0.12
17	NP_253284.1		PA4594 probable ATP-binding component of ABC transporter	H220D	5.11×10^{-18}	0.1991
18	NP_250663.1		pyrroloquinoline quinone biosynthesis protein F	H3R	6.543×10^{-18}	0.17
19	NP_251048.1	<i>proA</i>	PA2358	L2P	2.17×10^{-18}	19.34
20	NP_252696.1		gamma-glutamyl phosphate reductase	R275Q	6.07×10^{-14}	0.07
21	NP_250046.1	<i>wapB</i>	PA1355	C37G	1.67×10^{-13}	18.06
22	NP_249234.1		PA0543	S64P	2.32×10^{-13}	85.03
23	NP_249705.1		1,2-glucosyltransferase WapB	D12S	3.97×10^{-13}	91.89

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>p value</i>	<i>Odds ratio</i>
24	NP_249705.1	<i>wapB</i>	1,2-glucosyltransferase WapB	S25A	3.97×10^{-13}	91.89
25	NP_250046.1	<i>narG</i>	PA1355	S29G	8.22×10^{-13}	0
26	NP_252564.1		respiratory nitrate reductase alpha chain	A144V	1.26×10^{-12}	31.02
27	NP_249234.1	<i>cysH</i>	PA0543	A49T	1.30×10^{-12}	73.72
28	NP_249234.1		PA0543	A38V	2.12×10^{-12}	73.14
29	NP_250447.1		3'-phosphoadenosine-5'-phosphosulfate reductase	L2P	2.54×10^{-12}	12.54
30	NP_249234.1		PA0543	A103T	2.90×10^{-12}	73.12
31	NP_249234.1	<i>ppc</i>	PA0543	R53W	2.96×10^{-12}	72.91
32	NP_249234.1		PA0543	L320V	2.96×10^{-12}	72.91
33	NP_249234.1		PA0543	G285A	2.97×10^{-12}	72.71
34	NP_249234.1		PA0543	P35S	3.26×10^{-12}	72.81
35	NP_249234.1		PA0543	D252A	3.27×10^{-12}	72.84
36	NP_249234.1		PA0543	S149L	3.36×10^{-12}	70.02
37	NP_249234.1		PA0543	G243S	3.59×10^{-12}	72.7
38	NP_249234.1		PA0543	V152I	3.99×10^{-12}	72.59
39	NP_249234.1		PA0543	L235P	4.63×10^{-12}	70.55
40	NP_249234.1		PA0543	P34V	4.81×10^{-12}	72.38
41	NP_249234.1		PA0543	V196G	4.81×10^{-12}	72.38
42	NP_252377.1		phosphoenolpyruvate carboxylase	T722M	5.56×10^{-12}	NA
43	NP_252377.1		phosphoenolpyruvate carboxylase	A399V	5.60×10^{-12}	NA
44	NP_252377.1		phosphoenolpyruvate carboxylase	V829S	5.60×10^{-12}	NA
45	NP_252019.1		PA3329	G86D	6.19×10^{-12}	46.07
46	NP_250181.1		PA1490 probable transcriptional regulator	S30Y	6.62×10^{-12}	78.34
47	NP_250181.1		PA1490 probable transcriptional regulator	S207A	6.63×10^{-12}	78.37
48	NP_249976.1		PA1490 probable transcriptional regulator	D3V	8.77×10^{-12}	18.67
49	NP_250181.1		PA1490 probable transcriptional regulator	R245Q	9.56×10^{-12}	77.93
50	NP_250181.1		PA1490 probable transcriptional regulator	T255A	9.56×10^{-12}	77.93
51	NP_250181.1		PA1490 probable transcriptional regulator	P256A	9.56×10^{-12}	77.93
52	NP_249912.1	<i>mutL</i>	PA1221	V11A	9.72×10^{-12}	11.21
53	NP_252019.1		PA3329	N206H	1.40×10^{-11}	46.27
54	NP_253633.1		DNA mismatch repair protein MutL	A406T	1.46×10^{-11}	46.27
55	NP_250181.1		PA1490 probable transcriptional regulator	E158D	2.05×10^{-11}	73.04

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>p value</i>	<i>Odds ratio</i>
56	NP_253633.1	<i>mutL</i>	DNA mismatch repair protein MutL	392 deletion	2.43×10^{-11}	9.09
57	NP_253053.1	<i>iciA</i>	inhibitor of chromosome initiation IciA	M1L	2.51×10^{-11}	11.62
58	NP_252377.1	<i>ppc</i>	phosphoenolpyruvate carboxylase	E788D	2.63×10^{-11}	NA
59	NP_253633.1	<i>mutL</i>	DNA mismatch repair protein MutL	A391V	2.76×10^{-11}	9.00
60	NP_253616.1		PA4929	C20R	3.28×10^{-11}	NA
61	NP_254231.1		PA5544	I611V	3.69×10^{-11}	NA
62	NP_253633.1	<i>mutL</i>	DNA mismatch repair protein MutL	S2N	3.76×10^{-11}	9.01
63	NP_253633.1	<i>mutL</i>	DNA mismatch repair protein MutL	R207Q	4.38×10^{-11}	9.10
64	NP_249762.1	<i>braF</i>	branched-chain amino acid transport protein BraF	A231T	4.48×10^{-11}	60.51
65	NP_253633.1	<i>mutL</i>	DNA mismatch repair protein MutL	E428D	5.00×10^{-11}	8.91
66	NP_253108.1	<i>ftsI</i>	penicillin-binding protein 3	R504C	5.18×10^{-11}	70.53
67	NP_252019.1		PA3329	L66F	5.34×10^{-11}	39.52
68	NP_252019.1		PA3329	T148A	5.34×10^{-11}	39.52
69	NP_252019.1		PA3329	E409G	5.45×10^{-11}	39.66
70	NP_253633.1	<i>mutL</i>	DNA mismatch repair protein MutL	T118A	5.73×10^{-11}	8.94
71	NP_249718.1	<i>amaB</i>	delta1-Piperideine-6-carboxylate dehydrogenase	P45S	5.74×10^{-11}	15.57
72	NP_249718.1	<i>amaB</i>	delta1-Piperideine-6-carboxylate dehydrogenase	E406A	5.74×10^{-11}	15.57
73	NP_252019.1		PA3329	R75Q	6.11×10^{-11}	40.98
74	NP_252019.1		PA3329	A181S	6.54×10^{-11}	40.00
75	NP_252019.1		PA3329	V71L	6.99×10^{-11}	40.65
76	NP_251234.1		PA2544	T33S	8.24×10^{-11}	5.24
77	NP_249718.1	<i>amaB</i>	delta1-Piperideine-6-carboxylate dehydrogenase	S14L	9.72×10^{-11}	15.63
78	NP_249718.1	<i>amaB</i>	delta1-Piperideine-6-carboxylate dehydrogenase	G71R	9.95×10^{-11}	15.36
79	NP_249718.1	<i>amaB</i>	delta1-Piperideine-6-carboxylate dehydrogenase	N25H	1.23×10^{-10}	15.36

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>p value</i>	<i>Odds ratio</i>
80	NP_249718.1	<i>amaB</i>	delta1-Piperideine-6-carboxylate dehydrogenase	R49C	1.23×10^{-10}	15.36
81	NP_249718.1	<i>amaB</i>	delta1-Piperideine-6-carboxylate dehydrogenase	K340R	1.23×10^{-10}	15.36
82	NP_249976.1		PA1285 probable transcriptional regulator	R5K	1.24×10^{-10}	NA
83	NP_249718.1	<i>amaB</i>	delta1-Piperideine-6-carboxylate dehydrogenase	V357I	1.24×10^{-10}	15.34
84	NP_249718.1	<i>amaB</i>	delta1-Piperideine-6-carboxylate dehydrogenase	Q379E	1.35×10^{-10}	15.30
85	NP_250038.1		PA1347 probable transcriptional regulator	I6T	1.59×10^{-10}	21.01
86	NP_250038.1		PA1347 probable transcriptional regulator	Q30E	1.59×10^{-10}	21.01
87	NP_250038.1		PA1347 probable transcriptional regulator	D97G	1.87×10^{-10}	20.87
88	NP_250038.1		PA1347 probable transcriptional regulator	N40T	1.99×10^{-10}	20.84
89	NP_249718.1	<i>amaB</i>	delta1-Piperideine-6-carboxylate dehydrogenase	A253D	2.16×10^{-10}	15.22
90	NP_251593.1	<i>cobJ</i>	precorrin-3 methylase CobJ	T4A	2.20×10^{-10}	5.08
91	NP_252956.1	<i>fusA1</i>	elongation factor G	D588G	2.45×10^{-10}	NA
92	NP_251604.1		probable permease of ABC transporter	A281V	2.46×10^{-10}	NA
93	NP_253552.1	<i>ureA</i>	urease gamma subunit	E67G	2.54×10^{-10}	NA
94	NP_251108.1		PA2418	A47T	3.65×10^{-10}	15.13
95	NP_249140.1		PA0449	W38R	3.75×10^{-10}	26.32
96	NP_249140.1		PA0449	G39S	3.75×10^{-10}	26.32
97	NP_252584.1		PA3895 probable transcriptional regulator	P316L	4.36×10^{-10}	16.44
98	NP_249005.1		L-cysteine transporter of ABC system FliY	K139R	4.39×10^{-10}	NA
99	NP_251108.1		PA2418	W96R	4.59×10^{-10}	14.80
100	NP_253532.1	<i>dipZ</i>	thiol:disulfide interchange protein DipZ	I451V	5.38×10^{-10}	30.11

B.6. Dataset 2 treeWAS results

Table 23: SNPs identified with treeWAS from dataset 2, SNPs within core genes. SNPs with absolute simultaneous association scores greater than or equal to 26 are reported.

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Association score</i>	<i>p value</i>
1	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H3R	51	0
2	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H12R	50	0
3	NP_251980.1	<i>tle1</i>	Tle1	D168E	45	0
4	NP_253053.1	<i>iciA</i>	inhibitor of chromosome initiation IciA	M1L	44	0
5	NP_253957.1		PA5270	S5A	43	0
6	NP_250046.1		PA1355	F33S	42	0
7	NP_250046.1		PA1355	C37G	42	0
8	NP_251048.1		PA2358	L2P	42	0
9	NP_250442.1		PA1751	1 insertion	-41	0
10	NP_249912.1		PA1221	V11A	40	0
11	NP_250447.1	<i>cysH</i>	3'-phosphoadenosine-5'-phosphosulfate reductase	L2P	39	0
12	NP_248733.1		PA0043	M1D	38	0
13	NP_248733.1		PA0043	W4R	38	0
14	NP_252820.1		PA4131 probable iron-sulfur protein	R48P	37	0
15	NP_249491.1		PA0800	H78R	36	0
16	NP_249538.1		diguanylate cyclase	D34G	36	0
17	NP_250386.1	<i>pscP</i>	translocation protein in type III secretion	I83T	34	0
18	NP_250046.1		PA1355	S29G	33	0
19	NP_250737.1		PA2047 probable transcriptional regulator	M1V	33	0
20	NP_250737.1		PA2047 probable transcriptional regulator	V3A	33	0
21	NP_250737.1		PA2047 probable transcriptional regulator	T6A	33	0
22	NP_251234.1		PA2544	G20C	31	0
23	NP_251980.1	<i>tle1</i>	Tle1	N131S	29	0
24	NP_253462.1		PA4774	308 deletion	-29	0
25	NP_253462.1		PA4774	X350E	-29	0
26	NP_250386.1	<i>pscP</i>	translocation protein in type III secretion	L18P	28	0
27	NP_251980.1	<i>tle1</i>	Tle1	R92K	28	0
28	NP_251980.1	<i>tle1</i>	Tle1	I109F	28	0
29	NP_249705.1	<i>wapB</i>	1,2-glucosyltransferase WapB	S25A	27	0
30	NP_249976.1		PA1285 probable transcriptional regulator	D3V	27	0

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Association score</i>	<i>p value</i>
31	NP_251980.1	<i>tle1</i>	Tle1	P2S	27	0
32	NP_251980.1	<i>tle1</i>	Tle1	3 insertion	27	0
33	NP_251980.1	<i>tle1</i>	Tle1	F6V	27	0
34	NP_251980.1	<i>tle1</i>	Tle1	I8V	27	0
35	NP_251980.1	<i>tle1</i>	Tle1	T19P	27	0
36	NP_251980.1	<i>tle1</i>	Tle1	Y20L	27	0
37	NP_251980.1	<i>tle1</i>	Tle1	V53E	27	0
38	NP_251980.1	<i>tle1</i>	Tle1	A62T	27	0
39	NP_251980.1	<i>tle1</i>	Tle1	I63L	27	0
40	NP_251980.1	<i>tle1</i>	Tle1	I65V	27	0
41	NP_251980.1	<i>tle1</i>	Tle1	I94F	27	0
42	NP_251980.1	<i>tle1</i>	Tle1	D102S	27	0
43	NP_251980.1	<i>tle1</i>	Tle1	K111R	27	0
44	NP_251980.1	<i>tle1</i>	Tle1	F127Y	27	0
45	NP_252321.1		PA3631	377 deletion	-27	0
46	NP_252321.1		PA3631	X409L	-27	0
47	NP_253781.1		PA5094 probable ATP-binding component of ABC transporter	269 deletion	-27	0
48	NP_253781.1		PA5094 probable ATP-binding component of ABC transporter	X277Q	-27	0
49	NP_251980.1	<i>tle1</i>	Tle1	V149T	26	0
50	NP_251980.1	<i>tle1</i>	Tle1	N3E	26	0
51	NP_251980.1	<i>tle1</i>	Tle1	F4V	26	0
52	NP_251980.1	<i>tle1</i>	Tle1	G5T	26	0
53	NP_251980.1	<i>tle1</i>	Tle1	H7S	26	0
54	NP_251980.1	<i>tle1</i>	Tle1	T11E	26	0
55	NP_251980.1	<i>tle1</i>	Tle1	H12F	26	0
56	NP_251980.1	<i>tle1</i>	Tle1	V14K	26	0
57	NP_251980.1	<i>tle1</i>	Tle1	A15E	26	0
58	NP_251980.1	<i>tle1</i>	Tle1	G16R	26	0
59	NP_251980.1	<i>tle1</i>	Tle1	R17L	26	0
60	NP_251980.1	<i>tle1</i>	Tle1	D21E	26	0
61	NP_251980.1	<i>tle1</i>	Tle1	S22P	26	0
62	NP_251980.1	<i>tle1</i>	Tle1	K23S	26	0
63	NP_251980.1	<i>tle1</i>	Tle1	K24V	26	0
64	NP_251980.1	<i>tle1</i>	Tle1	E27K	26	0
65	NP_251980.1	<i>tle1</i>	Tle1	L30S	26	0
66	NP_251980.1	<i>tle1</i>	Tle1	K31R	26	0
67	NP_251980.1	<i>tle1</i>	Tle1	Q33V	26	0
68	NP_251980.1	<i>tle1</i>	Tle1	D35E	26	0
69	NP_251980.1	<i>tle1</i>	Tle1	V38K	26	0
70	NP_251980.1	<i>tle1</i>	Tle1	F39Y	26	0
71	NP_251980.1	<i>tle1</i>	Tle1	S40N	26	0
72	NP_251980.1	<i>tle1</i>	Tle1	V54S	26	0

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Association score</i>	<i>p value</i>
73	NP_251980.1	<i>tle1</i>	Tle1	G55S	26	0
74	NP_251980.1	<i>tle1</i>	Tle1	A57I	26	0
75	NP_251980.1	<i>tle1</i>	Tle1	T66S	26	0
76	NP_251980.1	<i>tle1</i>	Tle1	L67I	26	0
77	NP_251980.1	<i>tle1</i>	Tle1	F69Y	26	0
78	NP_251980.1	<i>tle1</i>	Tle1	K77R	26	0
79	NP_251980.1	<i>tle1</i>	Tle1	V83L	26	0
80	NP_251980.1	<i>tle1</i>	Tle1	S84P	26	0
81	NP_251980.1	<i>tle1</i>	Tle1	C87R	26	0
82	NP_251980.1	<i>tle1</i>	Tle1	I98V	26	0
83	NP_251980.1	<i>tle1</i>	Tle1	S100E	26	0
84	NP_251980.1	<i>tle1</i>	Tle1	D103V	26	0
85	NP_251980.1	<i>tle1</i>	Tle1	I104Q	26	0
86	NP_251980.1	<i>tle1</i>	Tle1	S106G	26	0
87	NP_251980.1	<i>tle1</i>	Tle1	R107L	26	0
88	NP_251980.1	<i>tle1</i>	Tle1	K125R	26	0
89	NP_251980.1	<i>tle1</i>	Tle1	M132L	26	0
90	NP_251980.1	<i>tle1</i>	Tle1	G136A	26	0
91	NP_251980.1	<i>tle1</i>	Tle1	E138A	26	0
92	NP_251980.1	<i>tle1</i>	Tle1	I144V	26	0
93	NP_251980.1	<i>tle1</i>	Tle1	L148I	26	0
94	NP_251980.1	<i>tle1</i>	Tle1	Q150R	26	0
95	NP_251980.1	<i>tle1</i>	Tle1	A154V	26	0
96	NP_251980.1	<i>tle1</i>	Tle1	F156Q	26	0
97	NP_251980.1	<i>tle1</i>	Tle1	Y157G	26	0
98	NP_251980.1	<i>tle1</i>	Tle1	L160T	26	0
99	NP_251980.1	<i>tle1</i>	Tle1	160 insertion	26	0
100	NP_251980.1	<i>tle1</i>	Tle1	K161E	26	0
101	NP_251980.1	<i>tle1</i>	Tle1	R163P	26	0
102	NP_251980.1	<i>tle1</i>	Tle1	K165L	26	0
103	NP_251980.1	<i>tle1</i>	Tle1	N167D	26	0
104	NP_251980.1	<i>tle1</i>	Tle1	V169S	26	0
105	NP_251980.1	<i>tle1</i>	Tle1	G171T	26	0

B.7. Dataset 2 random forest results

Table 24: SNPs identified with RF from dataset 2, SNPs within core genes. SNPs with importances greater than or equal to 0.002 are reported.

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Importance</i>
1	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H12R	0.14
2	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H3R	0.098
3	NP_254125.1		PA5438 probable transcriptional regulator	272 deletion	0.076

Rank	Accession	Gene name	Protein name	Mutation	Importance
4	NP_251831.1	<i>wbpM</i>	nucleotide sugar epimerase/dehydratase WbpM	V113P	0.049
5	NP_248708.1	<i>fnt</i>	methionyl-tRNA formyltransferase	I196V	0.04
6	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	K92R	0.039
7	NP_249976.1		PA1285 probable transcriptional regulator	D3V	0.015
8	NP_253053.1	<i>iciA</i>	inhibitor of chromosome initiation IciA	M1L	0.012
9	NP_250447.1	<i>cysH</i>	3'-phosphoadenosine-5'-phosphosulfate reductase	L2P	0.012
10	NP_248733.1		PA0043	W4R	0.01
11	NP_249939.1	<i>aprF</i>	Alkaline protease secretion outer membrane protein AprF precursor	T82A	0.009
12	NP_250442.1		PA1751	1 insertion	0.009
13	NP_251249.1		PA2559	A161S	0.008
14	NP_250319.1		PA1628 probable 3-hydroxyacyl-CoA dehydrogenase	R48Q	0.008
15	NP_249004.1		L-cysteine transporter of ABC system YecS	164 insertion	0.008
16	NP_251435.1		PA2745 probable hydrolase	L88G	0.007
17	NP_250108.1		PA1417 probable decarboxylase	T262A	0.007
18	NP_249286.1	<i>lptD</i>	LPS-assembly protein LptD	E812K	0.007
19	NP_251993.1		PA3303 probable major facilitator superfamily (MFS) transporter	H262R	0.006
20	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	C240G	0.006
21	NP_250310.1		PA1619 probable transcriptional regulator	I14V	0.005
22	NP_251980.1	<i>tle1</i>	Tle1	I109F	0.005
23	NP_251358		PA2668	5 insertion	0.005
24	NP_251232.1		PA2542	1130 insertion	0.004
25	NP_251315.1		PA2625	V84I	0.004
26	NP_249533.1		PA0842 probable glycosyl transferase	K251R	0.004
27	NP_252886.1	<i>bfiS</i>	BfiS	E431A	0.004
28	NP_249622.1	<i>pirA</i>	ferric enterobactin receptor PirA	A370T	0.004
29	NP_249286.1	<i>lptD</i>	LPS-assembly protein LptD	D593N	0.004
30	NP_252466.1	<i>xseA</i>	exodeoxyribonuclease VII large subunit	L325Q	0.004
31	NP_253191.1	<i>opdD</i>	Glycine-glutamate dipeptide porin OpdP	E30A	0.004
32	NP_248937.1		PA0246 probable major facilitator superfamily (MFS) transporter	Y43F	0.004
33	NP_251716.1		PA3026	E464Q	0.004

Rank	Accession	Gene name	Protein name	Mutation	Importance
34	NP_251210.1	<i>czcA</i>	Resistance-Nodulation-Cell Division (RND) divalent metal cation efflux transporter CzcA	R595K	0.004
35	NP_253957.1	<i>tse6</i>	PA5270	S5A	0.004
36	NP_248783.1		Tse6	387 deletion	0.004
37	NP_248891.1		PA0200	E64D	0.003
38	NP_253549.1	<i>tse6</i>	PA4862 probable ATP-binding component of ABC transporter	A174T	0.003
39	NP_251601.1		PA2911 probable TonB-dependent receptor	Y684D	0.003
40	NP_248783.1		Tse6	A392L	0.003
41	NP_253939.1		PA5252 probable ATP-binding component of ABC transporter	T225I	0.003
42	NP_253462.1		PA4774	X350E	0.003
43	NP_252350.1	<i>opdD</i>	PA3660 probable sodium/hydrogen antiporter	A14T	0.003
44	NP_248969.1		PA0278	V111A	0.003
45	NP_253191.1		Glycine-glutamate dipeptide porin OpdP	N31T	0.003
46	NP_252017.1		PA3327 probable non-ribosomal peptide synthetase	E402K	0.003
47	NP_251604.1		PA2914 probable permease of ABC transporter	I255V	0.003
48	NP_251115.1	<i>pvdG</i>	PvdG	D178E	0.003
49	NP_251358	<i>radC</i>	PA2668	R4L	0.003
50	NP_249925.1		PA1234	N39D	0.003
51	NP_254006.1		DNA repair protein RadC	D212G	0.003
52	NP_250687.1		PA1997 probable AMP-binding enzyme	A328T	0.003
53	NP_251593.1	<i>cobJ</i>	precorrin-3 methylase CobJ	T4A	0.003
54	NP_248764.1	<i>ppkA</i>	serine/threonine protein kinase PpkA	L295M	0.003
55	NP_253663.1	<i>aruH</i>	Arginine:Pyruvate Transaminas, AruH	R84Q	0.003
56	NP_252007.1	<i>pscK</i>	PA3317	S126N	0.003
57	NP_249290.1		PA0599	A20T	0.002
58	NP_250415.1		type III export protein PscK	E137D	0.002
59	NP_253413.1		two-component sensor CbrA	T16A	0.002
60	NP_250612.1	<i>cbrA</i>	PA1922 probable TonB-dependent receptor	V419A	0.002
61	NP_250774.1	<i>bkdB</i>	PA2084 probable asparagine synthetase	V118A	0.002
62	NP_251003		PA2313	H205O	0.002
63	NP_250806.1		PA2116	T148A	0.002
64	NP_250939.1		branched-chain alpha-keto acid dehydrogenase (lipoamide component)	S321G	0.002
65	NP_251749.1	<i>pelF</i>	PelF	N229S	0.002

Rank	Accession	Gene name	Protein name	Mutation	Importance
66	NP_251519.1		PA2829	G15A	0.002
67	NP_250692.1		PA2002	V457G	0.002
68	NP_251005.1		PA2315	V284I	0.002
69	NP_249124.1		PA0433	H116Q	0.002
70	NP_249633.1		PA0942 probable transcriptional regulator	D172A	0.002
71	NP_254011.1	<i>sphR</i>	Sphingosine-responsive Regulator, SphR	D347E	0.002
72	NP_251124.1		PA2434	A145T	0.002
73	NP_251005.1		PA2315	L306V	0.002
74	NP_250470.1		assimilatory nitrate reductase	T549S	0.002
75	NP_251358		PA2668	R4I	0.002
76	NP_251157.1	<i>foxR</i>	Anti-sigma factor FoxR	A28G	0.002
77	NP_252126.1		PA3436	H92R	0.002
78	NP_253777.1	<i>vgrG5</i>	VgrG5	A242T	0.002
79	NP_253179.1	<i>magD</i>	MagD	M1009V	0.002
80	NP_251602.1		PA2912 probable ATP-binding component of ABC transporter	S185G	0.002
81	NP_249292.1	<i>agtR</i>	two-component response regulator, AgtR	1 deletion	0.002
82	NP_249158.1		PA0467	G140A	0.002
83	NP_251114.1	<i>pvdL</i>	PvdL	V3938I	0.002
84	YP_008719781.1		PA4638	F87R	0.002
85	NP_253669		probable two-component sensor	N904S	0.002
86	NP_250356.1	<i>fla2</i>	Fha2	G277S	0.002
87	NP_250705.1	<i>liuA</i>	putative isovaleryl-CoA dehydrogenase	A179P	0.002
88	NP_249019.1	<i>aaaA</i>	arginine-specific autotransporter of Pseudomonas aeruginosa, AaaA	H516R	0.002
89	NP_250023.1		PA1332	C48R	0.002
90	NP_250630.1		PA1940	T284A	0.002
91	NP_252454.1		PA3765	S62R	0.002
92	NP_252030.1		PA3340	S181N	0.002
93	NP_253231.1	<i>lepA</i>	Pseudomonas aeruginosa-derived large extracellular protease, LepA	S550N	0.002
94	NP_251970.1	<i>oprO</i>	Pyrophosphate-specific outer membrane porin OprO precursor	F86Y	0.002
95	NP_251813.1		PA3123	S37T	0.002
96	NP_251980.1	<i>tle1</i>	Tle1	N131S	0.002
97	NP_249399.1		PA0708 probable transcriptional regulator	G181S	0.002
98	NP_249104.1	<i>chpA</i>	component of chemotactic signal transduction system	V594L	0.002
99	NP_249104.1	<i>chpA</i>	component of chemotactic signal transduction system	E825A	0.002
100	NP_252913.1		PA4223 probable ATP-binding component of ABC transporter	S484R	0.002
101	NP_252236.1	<i>algX</i>	alginate biosynthesis protein AlgX	E201D	0.002
102	NP_251766.1		PA3076	D42A	0.002

Rank	Accession	Gene name	Protein name	Mutation	Importance
103	NP_251204.1	<i>antC</i>	anthranilate dioxygenase reductase	D87N	0.002
104	NP_249109.1		PA0418	T455R	0.002

B.8. Dataset 2 support vector machine results

Table 25: SNPs identified with SVM from dataset 2, SNPs within core genes. Top 100 SNPs with absolute hyperplane coefficients greater than 0.03 are reported.

Rank	Accession	Gene name	Protein name	Mutation	Hyperplane coefficient
1	NP_251083.1		PA2393 putative dipeptidase	G22A	0.5017
2	NP_251083.1		PA2393 putative dipeptidase	21 insertion	-0.5011
3	NP_251083.1		PA2393 putative dipeptidase	G22V	-0.5008
4	NP_251083.1		PA2393 putative dipeptidase	22 insertion	0.4963
5	NP_249622.1	<i>pirA</i>	ferric enterobactin receptor PirA	A370T	-0.3394
6	NP_252366.1	<i>mexK</i>	MexK	E765A	0.3369
7	NP_252366.1	<i>mexK</i>	MexK	K694R	-0.2909
8	NP_249622.1	<i>pirA</i>	ferric enterobactin receptor PirA	A228V	0.2810
9	NP_249622.1	<i>pirA</i>	ferric enterobactin receptor PirA	V128A	0.2808
10	NP_253633.1	<i>mutL</i>	DNA mismatch repair protein MutL	E428D	-0.2808
11	NP_252612.1		PA3923	A137V	0.2803
12	NP_251391.1		PA2701 probable major facilitator superfamily (MFS) transporter	V304A	0.2802
13	NP_250322.1		PA1631 probable acyl-CoA dehydrogenase	D172N	-0.2801
14	NP_251747.1		PA3057	A72R	0.2572
15	NP_252156.1		PA3466 probable ATP-dependent RNA helicase	S4F	0.2422
16	NP_251831.1	<i>wbpM</i>	nucleotide sugar epimerase/dehydratase WbpM	I7G	0.1939
17	NP_251831.1	<i>wbpM</i>	nucleotide sugar epimerase/dehydratase WbpM	K8R	0.1863
18	NP_251858.1	<i>gyrA</i>	DNA gyrase subunit A	D87Y	-0.1763
19	NP_249064.1	<i>ftsY</i>	signal recognition particle receptor FtsY	120 deletion	-0.1527
20	NP_249004.1		L-cysteine transporter of ABC system YecS	164 insertion	0.1507
21	NP_250804.1		PA2114 probable major facilitator superfamily (MFS) transporter	A168T	0.1165
22	NP_249593.1		PA0902	G162D	0.1158
23	NP_253213.1		PA4523	A184P	0.1158
24	NP_249979.1		PA1288 probable outer membrane protein precursor	G176S	-0.1142
25	NP_248764.1	<i>ppkA</i>	serine/threonine protein kinase PpkA	Q105L	0.1140

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Hyperplane coefficient</i>
26	NP_254125.1	<i>ppkA</i>	PA5438 probable transcriptional regulator	272 deletion	0.1026
27	NP_250890.1		Cyclic-guanylate-specific phosphodiesterase	A529T	-0.0962
28	NP_248764.1		serine/threonine protein kinase PpkA	L295M	0.0941
29	NP_250036.1	<i>ppkA</i>	PA1345	L17P	0.0905
30	NP_249860.1		PA1169 probable lipoxygenase	T137A	0.0851
31	NP_248764.1		serine/threonine protein kinase PpkA	A108V	0.0846
32	NP_253847.1		drug efflux transporter	V211I	-0.0789
33	NP_253847.1		drug efflux transporter	H421N	-0.0785
34	NP_249860.1		PA1169 probable lipoxygenase	I181L	0.0775
35	NP_249860.1		PA1169 probable lipoxygenase	R203G	0.0774
36	NP_251367.1		PA2677 probable type II secretion protein	M12V	0.0765
37	NP_249860.1		PA1169 probable lipoxygenase	G84A	0.0737
38	NP_253847.1		drug efflux transporter	T86A	-0.0734
39	NP_252321.1		PA3631	377 deletion	-0.0702
40	NP_252321.1		PA3631	X409L	-0.0698
41	NP_250765.1		PA2075	Q427L	-0.0696
42	NP_253462.1		PA4774	308 deletion	-0.0673
43	NP_253462.1		PA4774	X350E	-0.0673
44	NP_251367.1		PA2677 probable type II secretion protein	P10L	-0.0668
45	NP_249109.1		PA0418	T8P	-0.0664
46	NP_249064.1	<i>ftsY</i>	signal recognition particle receptor FtsY	121 deletion	0.0662
47	NP_251980.1	<i>tle1</i>	Tle1	D168E	0.0611
48	NP_253373.1		PA4684	407 deletion	-0.0610
49	NP_253373.1	<i>iciA</i>	PA4684	X433L	-0.0610
50	NP_253053.1		inhibitor of chromosome initiation IciA	M1L	0.0606
51	NP_253651.1	<i>parC</i>	topoisomerase IV subunit A	S87L	-0.0599
52	NP_249988.1		PA1297 probable metal transporter	172 insertion	-0.0599
53	NP_251124.1	<i>tpm</i>	PA2434	A145T	-0.0595
54	NP_251522.1		thiopurine methyltransferase	H82R	0.0590
55	NP_250442.1		PA1751	1 insertion	-0.0584
56	NP_250447.1	<i>cysH</i>	3'-phosphoadenosine-5'-phosphosulfate reductase	L2P	0.0584
57	NP_252681.1	<i>sltB3</i>	SltB3	H240Y	-0.0564
58	NP_251048.1		PA2358	L2P	0.0556

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Hyperplane coefficient</i>
59	NP_250755.1	<i>pcoA</i>	copper resistance protein A precursor	V401M	-0.0551
60	NP_253284.1		PA4594 probable ATP-binding component of ABC transporter	G203S	-0.0550
61	NP_253231.1	<i>lepA</i>	Pseudomonas aeruginosa-derived large extracellular protease, LepA	N424S	0.0547
62	NP_248840.1		anti-sigma factor	E12D	0.0543
63	NP_250124.1		PA1433	M476L	0.0542
64	NP_251519.1		PA2829	G15A	-0.0540
65	NP_249538.1		diguanylate cyclase	D34G	0.0534
66	NP_248783.1	<i>tse6</i>	Tse6	387 deletion	-0.0532
67	NP_248783.1	<i>tse6</i>	Tse6	A392L	-0.0532
68	NP_250025.1		PA1334 probable oxidoreductase	T7A	-0.0532
69	NP_248783.1	<i>tse6</i>	Tse6	R388L	0.0529
70	NP_248783.1	<i>tse6</i>	Tse6	388 deletion	0.0529
71	NP_251351.1		PA2661	D255O	0.0524
72	NP_251100.1	<i>fpvF</i>	FpvF	I276V	-0.0523
73	NP_251052.1	<i>dotU3</i>	DotU3	X253A	0.0522
74	NP_250046.1		PA1355	F33S	0.0521
75	NP_249696.1		PA1005	M14I	0.0521
76	NP_250056.1		PA1365 probable siderophore receptor	P799L	0.0521
77	NP_251980.1	<i>tle1</i>	Tle1	I231V	0.0521
78	NP_250046.1		PA1355	C37G	0.0520
79	NP_251063.1	<i>vgrG3</i>	VgrG3	T46S	0.0518
80	NP_252454.1		PA3765	S62R	-0.0515
81	NP_252538.1		PA3849	A52T	0.0514
82	NP_252538.1		PA3849	E44A	0.0514
83	NP_249838.1		PA1147 probable amino acid permease	V396A	-0.0511
84	NP_249020.1		PA0329	N62S	-0.0511
85	NP_253377.1	<i>hitB</i>	iron (III)-transport system permease HitB	L345V	-0.0510
86	NP_250613.1		PA1923	E65D	-0.0510
87	NP_250126.1		PA1435 probable Resistance-Nodulation-Cell Division (RND) efflux membrane fusion protein precursor	L230P	0.0505
88	NP_250557.1		PA1866	G425A	0.0504
89	NP_250923.1	<i>pslC</i>	PslC	D173G	-0.0503
90	NP_253960.1		PA5273	G233O	0.0503
91	NP_249976.1		PA1285 probable transcriptional regulator	D3V	0.0501
92	NP_064724.1	<i>gyrB</i>	DNA gyrase subunit B	S466F	0.0498
93	NP_252235.1	<i>algG</i>	alginate-c5-mannuronan-epimerase AlgG	G58S	0.0496

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Hyperplane coefficient</i>
94	NP_253284.1		PA4594 probable ATP-binding component of ABC transporter	Q5R	-0.0496
95	NP_251257.1		PA2567	G487S	-0.0496
96	NP_249912.1		PA1221	V11A	0.0494
97	NP_250290.1		PA1599 probable transcriptional regulator	D37E	-0.0493
98	NP_250127.1		PA1436 probable Resistance-Nodulation-Cell Division (RND) efflux transporter	T93S	0.0492
99	NP_253170.1	<i>mreC</i>	rod shape-determining protein MreC	S296O	0.0492
100	NP_250126.1		PA1435 probable Resistance-Nodulation-Cell Division (RND) efflux membrane fusion protein precursor	D325A	0.0490

B.9. Dataset 3 PLINK results

Table 26: Genes and SNPs identified with PLINK from dataset 3, combined gene presence/absence and SNPs within core genes. Top 100 variants with p values less than 1×10^{-8} are reported. 2640 genes had p values less than 1×10^{-8} .

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>p value</i>	<i>Odds ratio</i>
1	—		G5 domain containing protein		2.677×10^{-60}	24.43
2	NP_252650.1		PA3961 probable ATP-dependent helicase	A8S	1.59×10^{-49}	0.09
3	NP_252650.1		PA3961 probable ATP-dependent helicase	E214D	1.59×10^{-49}	0.09
4	NP_252777.1		PA4088 probable aminotransferase	L208P	3.663×10^{-49}	9.21
5	NP_253497.1	<i>fdhE</i>	FdhE protein	A235T	1.793×10^{-45}	10.55
6	NP_254099.1		PA5412	A68V	2.795×10^{-44}	11.09
7	NP_250624.1		PA1934	S99T	5.353×10^{-44}	12.06
8	NP_252274.1	<i>glpD</i>	glycerol-3-phosphate dehydrogenase	R274H	8.968×10^{-44}	0.10
9	NP_252276.1		PA3586 probable hydrolase	T102A	8.968×10^{-44}	0.10
10	NP_252172.1	<i>metG</i>	methionyl-tRNA synthetase	V76A	3.895×10^{-40}	0.11
11	NP_252172.1	<i>metG</i>	methionyl-tRNA synthetase	E659Q	3.895×10^{-40}	0.11
12	NP_251087.1	<i>pvdE</i>	pyoverdine biosynthesis protein PvdE	E327S	5.119×10^{-40}	0.11
13	NP_251087.1	<i>pvdE</i>	pyoverdine biosynthesis protein PvdE	S394E	5.119×10^{-40}	0.11

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>p value</i>	<i>Odds ratio</i>
14	NP_253584.1	<i>hasI</i>	TonB-dependent receptor	L14P	3.371×10^{-39}	2077
15	NP_251968.1		PA3278	A99V	3.106×10^{-37}	NA
16	NP_252100.1		HasI	R112H	1.871×10^{-36}	NA
17	–		hypothetical protein		2.769×10^{-36}	30.01
18	–	<i>amiR</i>	hypothetical protein		6.071×10^{-36}	157.2
19	NP_252053.1		aliphatic amidase	D163N	1.827×10^{-35}	2011
20	–		regulator			
21	–		terminase		4.339×10^{-35}	19.38
22	NP_253011.1		hypothetical protein		1.081×10^{-34}	21.71
23	NP_253481.1		PA4321		9.14×10^{-34}	0.13
24	NP_254235.1		PA4793	R84G	1.119×10^{-33}	7.16
			PA5548 probable major facilitator superfamily (MFS) transporter	V27I	6.548×10^{-33}	169.20
25	NP_253584.1		TonB-dependent receptor	D269N	1.476×10^{-32}	NA
26	NP_249248.1		PA0557	Q176K	3.887×10^{-32}	0.18
27	–		methyltransferase		1.478×10^{-31}	39.57
28	NP_250726.1		PA2036		4.727×10^{-31}	24.37
29	NP_253058.1	<i>lptD</i>	PA4368	L145M	4.823×10^{-31}	8.27
30	NP_249286.1		LPS-assembly protein	I888V	8.87×10^{-31}	10.07
			LptD			
31	NP_248957.1		delta-aminovaleate aminotransferase	A289V	1.016×10^{-30}	110.20
32	NP_253508.1		PA4821 probable transporter	Q408K	5.19×10^{-30}	16.46
33	–		hypothetical protein		8.94×10^{-30}	25.28
34	NP_253921.1		PA5234 probable oxidoreductase	I30V	2.442×10^{-29}	30.44
35	NP_252875.1		PA4186		3.38×10^{-29}	85.36
36	NP_252110.1	<i>trmA</i>	PA3420 probable transcriptional regulator	T193A	5.076×10^{-29}	14.51
37	NP_253408.1		tRNA (uracil-5-)-methyltransferase	S292N	1.783×10^{-28}	21.64
38	NP_251442.1		PA2752	R36H	6.795×10^{-28}	0.07
39	NP_248783.1		Tse6	F422Y	9.084×10^{-28}	47.61
40	NP_249248.1	<i>tse6</i>	PA0557	P19L	2.338×10^{-27}	0.21
41	NP_250087.1		PA1396 probable two-component sensor	D509N	3.426×10^{-27}	10.96
42	–		type I-E CRISPR-associated protein Cas5/CasD		1.165×10^{-26}	18.91
43	NP_250086.1		PA1395	G16S	1.932×10^{-26}	9.15
44	NP_250699.1	<i>hmgA</i>	homogentisate 1,2-dioxygenase	A250G	2.086×10^{-26}	5.31
45	NP_249709.1	<i>thiC</i>	PA1018	E159K	2.353×10^{-26}	18.26
46	NP_253660.1		thiamin biosynthesis protein ThiC	D447N	2.413×10^{-26}	20.06

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>p value</i>	<i>Odds ratio</i>
47	NP_252283.1		PA3593 probable acyl-CoA dehydrogenase	P451S	4.82×10^{-26}	27.32
48	NP_254178.1		PA5491 probable cytochrome	T67S	7.193×10^{-26}	14.55
49	NP_252534.1		PA3845 probable transcriptional regulator	A50T	7.288×10^{-26}	27.23
50	NP_253888.1		PA5201	D137N	1.131×10^{-25}	11.29
51	NP_253148.1		PA4458		1.194×10^{-25}	18.06
52	–		elongation factor Tu (pseudogene)		2.797×10^{-25}	5.17
53	–		hypothetical protein		4.752×10^{-25}	93.00
54	–		protein kinase		4.752×10^{-25}	93.00
55	–		hypothetical protein		1.178×10^{-24}	1414
56	NP_251264.1	<i>alkB1</i>	alkane-1-monooxygenase	V325A	1.719×10^{-24}	32.01
57	NP_253151.1	<i>lptB</i>	Lipopolysaccharide export system ATP-binding protein LptB		2.772×10^{-24}	NA
58	NP_251706.1		PA3016		3.144×10^{-24}	6.14
59	–		usher CupC3 (pseudogene)		3.576×10^{-24}	0.21
60	NP_254209.1	<i>pauA6</i>	Glutamylpolyamine synthetase	Q226V	6.795×10^{-24}	NA
61	NP_248913.1		PA0222	Y8C	1.224×10^{-23}	24.70
62	NP_253551.1	<i>ureD</i>	urease accessory protein	A37S	2.997×10^{-23}	NA
63	NP_248982.1	<i>oprE</i>	Anaerobically-induced outer membrane porin OprE precursor	440 insertion	5.95×10^{-23}	12.11
64	NP_251512.1		PA2822	I120L	8.727×10^{-23}	11.69
65	NP_253413.1	<i>cbrA</i>	two-component sensor CbrA	V673A	9.505×10^{-23}	57.97
66	NP_253590.1		PA4903 probable major facilitator superfamily (MFS) transporter	R220H	1.085×10^{-22}	58.68
67	NP_249693.1	<i>phnB</i>	anthranilate synthase component II	A73V	1.237×10^{-22}	NA
68	NP_250047.1		PA1356		1.237×10^{-22}	NA
69	NP_251873.1	<i>zwf</i>	glucose-6-phosphate 1-dehydrogenase	E109D	1.237×10^{-22}	NA
70	NP_252214.1	<i>gloA1</i>	lactoylglutathione lyase	D87N	1.237×10^{-22}	NA
71	NP_252769.1		PA4080 probable response regulator	N149T	1.237×10^{-22}	NA
72	–		hypothetical protein		1.237×10^{-22}	NA
73	NP_253616.1		PA4929	A402S	1.425×10^{-22}	11.52
74	NP_251114.1	<i>pvdL</i>	PvdL	R1655Q	1.49×10^{-22}	NA
75	NP_248708.1	<i>fnt</i>	methionyl-tRNA formyltransferase	A188V	1.805×10^{-22}	78.48
76	NP_249401.1	<i>gloA2</i>	lactoylglutathione lyase	A89D	2.105×10^{-22}	7.79

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>p value</i>	<i>Odds ratio</i>
77	NP_251316.1	<i>trmU</i>	tRNA methyltransferase		3.785×10^{-22}	11.3
78	NP_253584.1		TonB-dependent receptor	M497T	4.648×10^{-22}	108.2
79	NP_253541.1	<i>purH</i>	phosphoribosylaminoimidazole formyltransferase	A116W N123	1.923×10^{-22}	NA
80	NP_252516.1	<i>lptG</i>	Lipopolysaccharide export system permease protein LptG	218 insertion	5.502×10^{-22}	100.10
81	NP_253113.1		PA4423	E219D	6.694×10^{-22}	11.20
82	NP_250443.1		PA1752	Y295H	7.166×10^{-22}	12.28
83	NP_253127.1		PA4437	A67S	7.308×10^{-22}	24.73
84	NP_251939.1		PA3249 probable transcriptional regulator		1.341×10^{-21}	22.50
85	NP_250885.1	<i>hcnC</i>	hydrogen cyanide synthase HcnC	P209L	1.35×10^{-21}	32.45
86	NP_250865.1		PA2175	R91W	2.189×10^{-21}	64.66
87	NP_252485.1		PA3796	102 insertion	2.313×10^{-21}	9.11
88	NP_253669		PA4982 probable two-component sensor	A825V	2.314×10^{-21}	11.21
89	NP_253135.1		PA4445	A54V	2.519×10^{-21}	NA
90	NP_251911.1	<i>csaA</i>	CsaA protein	V34I	2.644×10^{-21}	11.68
91	NP_253403.1		PA4715 probable aminotransferase	A339T	2.844×10^{-21}	7.18
92	NP_252623.1		PA3934	E156A	3.797×10^{-21}	16.65
93	NP_252472.1		PA3783	R68L	3.948×10^{-21}	NA
94	NP_252438.1		PA3749 probable major facilitator superfamily (MFS) transporter		4.551×10^{-21}	18.45
95	NP_253423.1		PA4735	A299V	7.268×10^{-21}	15.58
96	NP_253397.1	<i>phuS</i>	PhuS	Q133H	7.275×10^{-21}	1207
97	NP_251076.1	<i>pvdA</i>	L-ornithine N5-oxygenase	E369D	1.12×10^{-20}	20.79
98	NP_253584.1		TonB-dependent receptor	Q742R	1.136×10^{-20}	9.00
99	NP_251948.1		PA3258	G285S	1.29×10^{-20}	70.55
100	NP_249248.1		PA0557	H191Q	1.494×10^{-20}	NA

B.10. Dataset 3 treeWAS results

Table 27: Genes and SNPs identified with treeWAS from dataset 3, combined gene presence/absence and SNPs within core genes. Variants with absolute simultaneous association scores greater than or equal to 26 are reported.

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Association score</i>	<i>p value</i>
1	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H3R	50.5	0
2	NP_250018.1		PA1327 probable protease		-50	0

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Association score</i>	<i>p value</i>
3	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H12R	49.5	0
4	NP_249374.1	<i>hxcY</i>	HxcY		-49	0
5	NP_250230.1		PA1539		-48	0
6	NP_250420.1		PA1729		-48	0
7	NP_249865.1	<i>napA</i>	periplasmic nitrate reductase protein NapA		-46	0
8	NP_250376.1	<i>masA</i>	enolase-phosphatase E-1		-46	0
9	NP_251980.1	<i>tle1</i>	Tle1	D168E	45	0
10	NP_251618.1		PA2928		-45	0
11	NP_253053.1	<i>iciA</i>	inhibitor of chromosome initiation IciA	M1L	44	0
12	NP_248888.1	<i>tonB2</i>	TonB2		-44	0
13	NP_253957.1		PA5270	S5A	43	0
14	NP_250046.1		PA1355	F33S	42	0
15	NP_250046.1		PA1355	C37G	42	0
16	NP_251048.1		PA2358	L2P	42	0
17	NP_064723.1	<i>recF</i>	RecF protein		-42	0
18	NP_250442.1		PA1751	1 insertion	-41	0
19	NP_249912.1		PA1221	V11A	40	0
20	—		hypothetical protein		40	0
21	NP_249603.1		PA0912		-40	0
22	NP_250447.1	<i>cysH</i>	3'-phosphoadenosine-5'-phosphosulfate reductase	L2P	39	0
23	NP_248733.1		PA0043	M1D	38	0
24	NP_248733.1		PA0043	W4R	38	0
25	NP_252820.1		PA4131 probable iron-sulfur protein	R48P	37	0
26	NP_249491.1		PA0800	H78R	36	0
27	NP_249538.1		diguanylate cyclase	D34G	36	0
28	—		hypothetical protein		35	0
29	NP_251800.1		PA3110		-35	0
30	NP_253724.1		PA5037		-35	0
31	NP_250386.1	<i>pscP</i>	translocation protein in type III secretion	I83T	34	0
32	NP_250046.1		PA1355	S29G	33	0
33	NP_250737.1		PA2047 probable transcriptional regulator	M1V	33	0
34	NP_250737.1		PA2047 probable transcriptional regulator	V3A	33	0
35	NP_250737.1		PA2047 probable transcriptional regulator	T6A	33	0
36	NP_250764.1		PA2074		33	0
37	NP_251981.1	<i>tli1</i>	Tli1		32	0
38	NP_251234.1		PA2544	G20C	31	0
39	—		hypothetical protein		31	0

Rank	Accession	Gene name	Protein name	Mutation	Association score	p value
40	–		putative rubredoxin reductase		31	0
41	–		hypothetical protein		30	0
42	NP_251980.1	<i>tle1</i>	Tle1	N131S	29	0
43	–		glyA		29	0
44	–		ABC transporter substrate-binding protein		29	0
45	NP_253462.1		PA4774	308 deletion	-28.5	0
46	NP_253462.1		PA4774	X350E	-28.5	0
47	NP_250386.1	<i>pscP</i>	translocation protein in type III secretion	L18P	28	0
48	NP_251980.1	<i>tle1</i>	Tle1	R92K	28	0
49	NP_251980.1	<i>tle1</i>	Tle1	I109F	28	0
50	–		mammalian cell entry protein (pseudogene)		28	0
51	–		hypothetical protein		28	0
52	–		hypothetical protein		28	0
53	–		hypothetical protein (pseudogene)		28	0
54	NP_249705.1	<i>wapB</i>	1,2-glucosyltransferase WapB	S25A	27	0
55	NP_249976.1		PA1285 probable transcriptional regulator	D3V	27	0
56	NP_251980.1	<i>tle1</i>	Tle1	P2S	27	0
57	NP_251980.1	<i>tle1</i>	Tle1	3 insertion	27	0
58	NP_251980.1	<i>tle1</i>	Tle1	F6V	27	0
59	NP_251980.1	<i>tle1</i>	Tle1	I8V	27	0
60	NP_251980.1	<i>tle1</i>	Tle1	T19P	27	0
61	NP_251980.1	<i>tle1</i>	Tle1	Y20L	27	0
62	NP_251980.1	<i>tle1</i>	Tle1	V53E	27	0
63	NP_251980.1	<i>tle1</i>	Tle1	A62T	27	0
64	NP_251980.1	<i>tle1</i>	Tle1	I63L	27	0
65	NP_251980.1	<i>tle1</i>	Tle1	I65V	27	0
66	NP_251980.1	<i>tle1</i>	Tle1	I94F	27	0
67	NP_251980.1	<i>tle1</i>	Tle1	D102S	27	0
68	NP_251980.1	<i>tle1</i>	Tle1	K111R	27	0
69	NP_251980.1	<i>tle1</i>	Tle1	F127Y	27	0
70	NP_253781.1		PA5094 probable ATP-binding component of ABC transporter	269 deletion	-27	0
71	NP_253781.1		PA5094 probable ATP-binding component of ABC transporter	X277Q	-27	0
72	–		hypothetical protein		27	0
73	NP_248731.1		PA0041 probable hemagglutinin		-27	0

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Association score</i>	<i>p value</i>
74	NP_250105.1		PA1414		27	0
75	NP_250632.1		PA1942		27	0
76	NP_252321.1		PA3631	377 deletion	-26.5	0
77	NP_252321.1		PA3631	X409L	-26.5	0
78	NP_251980.1	<i>tle1</i>	Tle1	V149T	26	0
79	NP_251980.1	<i>tle1</i>	Tle1	N3E	26	0
80	NP_251980.1	<i>tle1</i>	Tle1	F4V	26	0
81	NP_251980.1	<i>tle1</i>	Tle1	G5T	26	0
82	NP_251980.1	<i>tle1</i>	Tle1	H7S	26	0
83	NP_251980.1	<i>tle1</i>	Tle1	T11E	26	0
84	NP_251980.1	<i>tle1</i>	Tle1	H12F	26	0
85	NP_251980.1	<i>tle1</i>	Tle1	V14K	26	0
86	NP_251980.1	<i>tle1</i>	Tle1	A15E	26	0
87	NP_251980.1	<i>tle1</i>	Tle1	G16R	26	0
88	NP_251980.1	<i>tle1</i>	Tle1	R17L	26	0
89	NP_251980.1	<i>tle1</i>	Tle1	D21E	26	0
90	NP_251980.1	<i>tle1</i>	Tle1	S22P	26	0
91	NP_251980.1	<i>tle1</i>	Tle1	K23S	26	0
92	NP_251980.1	<i>tle1</i>	Tle1	K24V	26	0
93	NP_251980.1	<i>tle1</i>	Tle1	E27K	26	0
94	NP_251980.1	<i>tle1</i>	Tle1	L30S	26	0
95	NP_251980.1	<i>tle1</i>	Tle1	K31R	26	0
96	NP_251980.1	<i>tle1</i>	Tle1	Q33V	26	0
97	NP_251980.1	<i>tle1</i>	Tle1	D35E	26	0
98	NP_251980.1	<i>tle1</i>	Tle1	V38K	26	0
99	NP_251980.1	<i>tle1</i>	Tle1	F39Y	26	0
100	NP_251980.1	<i>tle1</i>	Tle1	S40N	26	0
101	NP_251980.1	<i>tle1</i>	Tle1	V54S	26	0
102	NP_251980.1	<i>tle1</i>	Tle1	G55S	26	0
103	NP_251980.1	<i>tle1</i>	Tle1	A57I	26	0
104	NP_251980.1	<i>tle1</i>	Tle1	T66S	26	0
105	NP_251980.1	<i>tle1</i>	Tle1	L67I	26	0
106	NP_251980.1	<i>tle1</i>	Tle1	F69Y	26	0
107	NP_251980.1	<i>tle1</i>	Tle1	K77R	26	0
108	NP_251980.1	<i>tle1</i>	Tle1	V83L	26	0
109	NP_251980.1	<i>tle1</i>	Tle1	S84P	26	0
110	NP_251980.1	<i>tle1</i>	Tle1	C87R	26	0
111	NP_251980.1	<i>tle1</i>	Tle1	I98V	26	0
112	NP_251980.1	<i>tle1</i>	Tle1	S100E	26	0
113	NP_251980.1	<i>tle1</i>	Tle1	D103V	26	0
114	NP_251980.1	<i>tle1</i>	Tle1	I104Q	26	0
115	NP_251980.1	<i>tle1</i>	Tle1	S106G	26	0
116	NP_251980.1	<i>tle1</i>	Tle1	R107L	26	0
117	NP_251980.1	<i>tle1</i>	Tle1	K125R	26	0
118	NP_251980.1	<i>tle1</i>	Tle1	M132L	26	0
119	NP_251980.1	<i>tle1</i>	Tle1	G136A	26	0
120	NP_251980.1	<i>tle1</i>	Tle1	E138A	26	0

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Association score</i>	<i>p value</i>
121	NP_251980.1	<i>tle1</i>	Tle1	I144V	26	0
122	NP_251980.1	<i>tle1</i>	Tle1	L148I	26	0
123	NP_251980.1	<i>tle1</i>	Tle1	Q150R	26	0
124	NP_251980.1	<i>tle1</i>	Tle1	A154V	26	0
125	NP_251980.1	<i>tle1</i>	Tle1	F156Q	26	0
126	NP_251980.1	<i>tle1</i>	Tle1	Y157G	26	0
127	NP_251980.1	<i>tle1</i>	Tle1	L160T	26	0
128	NP_251980.1	<i>tle1</i>	Tle1	160 insertion	26	0
129	NP_251980.1	<i>tle1</i>	Tle1	K161E	26	0
130	NP_251980.1	<i>tle1</i>	Tle1	R163P	26	0
131	NP_251980.1	<i>tle1</i>	Tle1	K165L	26	0
132	NP_251980.1	<i>tle1</i>	Tle1	N167D	26	0
133	NP_251980.1	<i>tle1</i>	Tle1	V169S	26	0
134	NP_251980.1	<i>tle1</i>	Tle1	G171T	26	0

B.11. Dataset 3 random forest results

Table 28: Genes and SNPs identified with RF from dataset 3, combined gene presence/absence and SNPs within core genes. Variants with importances greater than or equal to 0.002 are reported.

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Importance</i>
1	NP_250764.1		PA2074		0.262
2	NP_259454.1	<i>mucA</i>	anti-sigma factor MucA		0.073
3	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H3R	0.051
4	NP_258888.1	<i>tonB2</i>	TonB2		0.038
5	NP_253724.1		PA5037		0.022
6	–		non-ribosomal peptide synthetase (pseudogene)		0.019
7	NP_252532.1		PA3843		0.01
8	–		mutL (pseudogene)		0.01
9	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H12R	0.01
10	NP_259620.1		two-component response regulator		0.009
11	–		5S ribosomal RNA		0.009
12	–		DNA-binding response regulator (pseudogene)		0.008
13	–		ffs		0.007
14	NP_253777.1	<i>vgrG5</i>	VgrG5	F699L	0.007
15	–		hypothetical protein		0.007
16	NP_252760.1		PA4071		0.007
17	–		hypothetical protein		0.006
18	–		hypothetical protein		0.006
19	NP_251831.1	<i>wbpM</i>	nucleotide sugar epimerase/dehydratase WbpM	V113P	0.005

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Importance</i>
20	NP_250828.1	<i>ligD</i>	Multifunctional non-homologous end joining protein LigD	S538G	0.005
21	–		hypothetical protein		0.005
22	–		hypothetical protein (pseudogene)		0.005
23	NP_249457.1	<i>mucD</i>	serine protease MucD precursor	V441I	0.005
24	NP_253893.1	<i>argE</i>	acetylornithine deacetylase	R221Q	0.005
25	NP_249711.1		PA1020 probable acyl-CoA dehydrogenase	S49T	0.005
26	NP_249004.1		L-cysteine transporter of ABC system YecS	164 insertion	0.004
27	–		hypothetical protein		0.004
28	NP_252030.1		PA3340	H311R	0.004
29	NP_252032.1		PA3342	A40P	0.004
30	NP_251980.1	<i>tle1</i>	Tle1	D168E	0.004
31	NP_249340.1	<i>trpG</i>	anthranilate synthase component II	D111N	0.004
32	NP_249933.1	<i>sprP</i>	SprP	E120D	0.004
33	NP_250710.1	<i>mexZ</i>	MexZ		0.004
34	NP_259117.1	<i>mexB</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux transporter MexB		0.004
35	NP_253820.1		PA5133		0.004
36	NP_250616.1		PA1926	V660L	0.004
37	NP_254125.1		PA5438 probable transcriptional regulator	272 deletion	0.004
38	NP_250959.1		PA2269	R204C	0.004
39	NP_250980.1	<i>gcd</i>	glucose dehydrogenase		0.004
40	NP_254723.1	<i>recF</i>	RecF protein		0.003
41	NP_249098.1	<i>gshB</i>	glutathione synthetase	R266L	0.003
42	NP_249438.1		PA0747 probable aldehyde dehydrogenase	V22I	0.003
43	NP_249973.1		PA1282 probable major facilitator superfamily (MFS) transporter	T219A	0.003
44	–		hypothetical protein		0.003
45	NP_253977.1		PA5290	M494T	0.003
46	NP_250152.1	<i>motD</i>	MotD	D107G	0.003
47	NP_249939.1	<i>aprF</i>	Alkaline protease secretion outer membrane protein AprF precursor	T82A	0.003
48	NP_252955.1	<i>tufA</i>	elongation factor Tu		0.003
49	–				0.003
50	NP_253236.1	<i>pilS</i>	two-component sensor PilS		0.003
51	NP_251134.1	<i>glyA2</i>	serine hydroxymethyltransferase		0.003
52	–		formate dehydrogenase-N subunit alpha (pseudogene)		0.003
53	YP_003933608.1		PA0457.1		0.003
54	–		tRNA-Ile		0.003
55	NP_249960.1		PA1269 probable transcriptional regulator	V95A	0.003
56	–		cystine ABC transporter substrate-binding protein (pseudogene)		0.003

Rank	Accession	Gene name	Protein name	Mutation	Importance
57	NP_250088.1		PA1397 probable two-component response regulator	D45G	0.003
58	–		protease (pseudogene)		0.003
59	NP_252899.1	<i>phzA1</i>	probable phenazine biosynthesis protein		0.003
60	NP_248956.1	<i>davD</i>	glutaric semialdehyde dehydrogenase	A338T	0.003
61	–				0.003
62	NP_250540.1		PA1849	V38T	0.003
63	NP_064724.1	<i>gyrB</i>	DNA gyrase subunit B	S466F	0.003
64	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	T206A	0.002
65	NP_250871.1		PA2181		0.002
66	NP_251359.1		PA2669	Q149E	0.002
67	NP_250244.1	<i>ccoO1</i>	Cytochrome c oxidase, cbb3-type, CcoO subunit		0.002
68	NP_249262.1		PA0571	R42H	0.002
69	NP_249110.1		PA0419		0.002
70	NP_252360.1		PA3670		0.002
71	NP_252831.1		PA4142 probable secretion protein	Q163R	0.002
72	–		hypothetical protein		0.002
73	NP_249170.1		PA0479 probable transcriptional regulator	G83D	0.002
74	NP_253393.1	<i>phuW</i>	PhuW	E125Q	0.002
75	NP_253525.1		PA4838	A166T	0.002
76	NP_252799.1	<i>ampC</i>	beta-lactamase precursor		0.002
77	NP_250404.1	<i>exsA</i>	transcriptional regulator ExsA		0.002
78	NP_252538.1		PA3849	M315T	0.002
79	NP_252405.1		PA3716	S374R	0.002
80	NP_249981.1		PA1290 probable transcriptional regulator	N175S	0.002
81	NP_251450.1	<i>oprQ</i>	OprQ	K56Q	0.002
82	NP_253191.1	<i>opdD</i>	Glycine-glutamate dipeptide porin OpdP	A19S	0.002
83	–		pilus assembly protein FimV (pseudogene)		0.002
84	NP_253350.1	<i>pagL</i>	Lipid A 3-O-deacylase		0.002
85	NP_250177.1	<i>bapF</i>	beta-peptidyl aminopeptidase	I264V	0.002
86	NP_254219.1		PA5532	252 insertion	0.002
87	NP_251044.1		PA2354 probable transcriptional regulator	T78N	0.002
88	NP_253462.1		PA4774	X350E	0.002
89	NP_251228.1		PA2538	I143V	0.002
90	–				0.002
91	NP_250018.1		PA1327 probable protease		0.002
92	NP_251858.1	<i>gyrA</i>	DNA gyrase subunit A	T83I	0.002
93	NP_249092.1		noncatalytic dihydroorotase-like protein	G130S	0.002

Rank	Accession	Gene name	Protein name	Mutation	Importance
94	NP_250515.1		PA1824	A148V	0.002
95	NP_252304.1		PA3614	S446G	0.002
96	NP_252801.1		PA4112 probable sensor/response regulator hybrid	A1110T	0.002
97	NP_249826.1		PA1135	G210D	0.002
98	NP_252644.1		PA3955	A191T	0.002
99	–		N-acetyltransferase GCN5		0.002
100	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	K92R	0.002
101	NP_250442.1		PA1751	A2V	0.002
102	NP_253231.1	<i>lepA</i>	Pseudomonas aeruginosa-derived large extracellular protease, LepA	D769N	0.002
103	NP_253340.1	<i>cupE4</i>	Pilin assembly chaperone CupE4	R228Q	0.002
104	NP_250144.1	<i>flhF</i>	flagellar biosynthesis protein FlhF	T84N	0.002
105	NP_251604.1		PA2914 probable permease of ABC transporter	G218C	0.002
106	NP_249416.1		PA0725 hypothetical protein of bacteriophage Pf1		0.002
107	NP_252716.1		PA4027	P293S	0.002
108	NP_248817.1		PA0127	Q125R	0.002
109	NP_248827.1		PA0137 probable permease of ABC transporter	S326T	0.002
110	NP_251831.1	<i>wbpM</i>	nucleotide sugar epimerase/dehydratase WbpM	S51G	0.002
111	NP_253996.1	<i>pauB4</i>	FAD-dependent oxidoreductase	T233A	0.002
112	NP_251800.1		PA3110		0.002
113	NP_249692.1	<i>phnA</i>	anthranilate synthase component I	D91A	0.002

B.12. Dataset 3 support vector machine results

Table 29: Genes and SNPs identified with SVM from dataset 3, combined gene presence/absence and SNPs within core genes. Top 100 variants with absolute hyperplane coefficients greater than 0.015 are reported.

Rank	Accession	Gene name	Protein name	Mutation	Hyperplane coefficient
1	NP_249454.1	<i>mucA</i>	anti-sigma factor MucA		-0.0432
2	–		dioxygenase		-0.0337
3	–		dioxygenase		0.0320
4	–		2,3-dihydro-3-hydroxyanthranilate isomerase		0.0315
5	NP_248888.1	<i>tonB2</i>	TonB2		-0.0296
6	NP_250036.1		PA1345	L17P	0.0284
7	NP_249116.1	<i>mexA</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux membrane fusion protein MexA precursor		-0.0281
8	NP_249004.1		L-cysteine transporter of ABC system YecS	164 insertion	0.0279

Rank	Accession	Gene name	Protein name	Mutation	Hyperplane coefficient
9	–	<i>oprB</i>	Glucose-selective porin OprB		0.0277
10	NP_250674.1	<i>exaC</i>	NAD ⁺ dependent aldehyde dehydrogenase ExaC		-0.0262
11	–		addiction module antitoxin RelB		0.0261
12	–		hypothetical protein		0.0255
13	NP_253000.1	<i>pctB</i>	chemotactic transducer PctB		0.0255
14	NP_253462.1		PA4774	308 deletion	-0.0249
15	NP_253462.1		PA4774	X350E	-0.0249
16	NP_249622.1	<i>pirA</i>	ferric enterobactin receptor PirA	A370T	-0.0244
17	–		integrating conjugative element protein		0.0239
18	NP_251876.1	<i>oprB</i>	Glucose/carbohydrate outer membrane porin OprB precursor		-0.0237
19	NP_250710.1	<i>mexZ</i>	MexZ		-0.0235
20	–		hypothetical protein		0.0227
21	NP_251835.1	<i>wbpL</i>	glycosyltransferase WbpL		0.0225
22	NP_252321.1		PA3631	377 deletion	-0.0224
23	NP_252321.1		PA3631	X409L	-0.0224
24	NP_248785.1		PA0095		0.0222
25	NP_254125.1		PA5438 probable transcriptional regulator	272 deletion	0.0219
26	–		hypothetical protein		-0.0216
27	–		hypothetical protein		0.0216
28	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H3R	0.0216
29	–		transcriptional regulator		0.0215
30	–		hypothetical protein		0.0215
31	–		hypothetical protein		-0.0215
32	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H12R	0.0215
33	–		type IV B pilus protein		0.0214
34	–		acetyl-CoA acetyltransferase		0.0214
35	–		hypothetical protein		0.0214
36	–		hypothetical protein		0.0213
37	–		group II intron reverse transcriptase/maturase		0.0211
38	–		protease (pseudogene)		0.0211
39	NP_253373.1		PA4684	407 deletion	-0.0211
40	NP_253373.1		PA4684	X433L	-0.0211
41	–		hypothetical protein		0.0210
42	–		endodeoxyribonuclease RusA		0.0208
43	–		hypothetical protein		-0.0203
44	–		hypothetical protein		0.0202
45	–		type IV pilus protein PilV		0.0202
46	–		hypothetical protein		0.0201

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Hyperplane coefficient</i>
47	–		toxin transporter		0.0201
48	–		hypothetical protein		0.0200
49	–		hypothetical protein		0.0200
50	NP_254102.1	<i>glyA1</i>	serine hydroxymethyltransferase		-0.0199
51	–		hypothetical protein		0.0197
52	–		hypothetical protein (pseudogene)		-0.0195
53	NP_251029.1		PA2339 probable binding-protein-dependent maltose/mannitol transport protein		-0.0195
54	NP_253781.1		PA5094 probable ATP-binding component of ABC transporter	269 deletion	-0.0194
55	NP_253781.1		PA5094 probable ATP-binding component of ABC transporter	X277Q	-0.0194
56	NP_251142		PA2452		-0.0194
57	–		porin (pseudogene)		-0.0194
58	–		twitching motility protein PilT		-0.0192
59	–		hypothetical protein		0.0189
60	–		hypothetical protein		-0.0188
61	NP_249374.1	<i>hxcY</i>	HxcY		-0.0188
62	–		hypothetical protein		-0.0187
63	–		hypothetical protein		-0.0187
64	–		type VI secretion protein ImpA (pseudogene)		-0.0186
65	–		hypothetical protein		-0.0186
66	–		bifunctional prephenate dehydrogenase/3- phosphoshikimate 1-carboxyvinyltransferase		-0.0183
67	–		hypothetical protein		-0.0183
68	NP_251023.1		PA2333 probable sulfatase		-0.0183
69	NP_251025.1		PA2335 probable TonB-dependent receptor		-0.0182
70	NP_250922.1	<i>pslB</i>	PslB		-0.0182
71	–		hypothetical protein		-0.0182
72	–		major exported protein		-0.0181
73	NP_250881.1	<i>exoY</i>	adenylate cyclase ExoY		-0.0181
74	–		acetyltransferase		0.0179
75	NP_251114.1	<i>pvdL</i>	PvdL	V3938I	-0.0179
76	–		hypothetical protein		0.0178
77	–		hypothetical protein		-0.0178
78	–		hypothetical protein		0.0178
79	NP_249552.1	<i>rbdA</i>	RbDA		-0.0178
80	–		hypothetical protein		-0.0178
81	NP_251148.1		PA2458		-0.0177
82	–		phosphodiesterase		-0.0177
83	–		cobalt transporter		-0.0177
84	–		23S ribosomal RNA		-0.0174
85	–		integrase		0.0174

Rank	Accession	Gene name	Protein name	Mutation	Hyperplane coefficient
86	—		hypothetical protein		0.0173
87	—		hypothetical protein		0.0173
88	—		membrane protein		-0.0173
89	—		hypothetical protein		-0.0173
90	—		hypothetical protein		-0.0173
91	—		DNA methyltransferase		0.0173
92	—		transporter		-0.0173
93	—		type IV B pilus protein		-0.0172
94	—		lipoprotein		0.0172
95	—		RNA polymerase subunit sigma (pseudogene)		0.0172
96	—		acetyl-CoA acetyltransferase		-0.0171
97	—		paraquat-inducible protein A		0.0171
98	—		hypothetical protein		-0.0171
99	NP_064723.1	<i>recF</i>	RecF protein		-0.0170
100	—		hypothetical protein		0.0170
101	—		hypothetical protein		0.0170

B.13. Dataset 4 PLINK results

Table 30: Genes and SNPs identified with PLINK from dataset 4, reduced dataset using features identified from random forest in datasets 1 and 2. Variants with p values less than 1×10^{-8} are reported.

Rank	Accession	Gene name	Protein name	Mutation	p value	Odds ratio
1	NP_248888.1	<i>tonB2</i>	TonB2		2.677×10^{-60}	24.43
2	NP_253781.1		PA5094 probable ATP-binding component of ABC transporter	X277Q	1.590×10^{-49}	0.09
3	NP_253957.1		PA5270	S5A	3.663×10^{-49}	9.21
4	—		hypothetical protein		1.793×10^{-45}	10.55
5	NP_253462.1		PA4774	X350E	8.968×10^{-44}	0.10
6	—		DNA-binding response regulator (pseudogene)		3.371×10^{-39}	2077
7	NP_253159.1		PA4469		3.106×10^{-37}	NA
8	NP_249374.1	<i>hxcY</i>	HxcY		2.769×10^{-36}	30.01
9	NP_249381.1	<i>pdtA</i>	phosphate depletion regulated TPS partner A, PdtA		1.081×10^{-34}	21.71
10	—		hypothetical protein		1.119×10^{-33}	7.16
11	—		hypothetical protein		6.548×10^{-33}	169.20
12	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H12R	3.887×10^{-32}	0.18
13	NP_064723.1	<i>recF</i>	RecF protein		1.478×10^{-31}	39.57
14	NP_249865.1	<i>napA</i>	periplasmic nitrate reductase protein NapA		4.727×10^{-31}	24.37

Rank	Accession	Gene name	Protein name	Mutation	p value	Odds ratio
15	–		pseudogene		5.190×10^{-30}	16.46
16	NP_249454.1	<i>mucA</i>	anti-sigma factor MucA		8.940×10^{-30}	25.28
17	NP_250018.1		PA1327 probable protease		3.380×10^{-29}	85.36
18	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H3R	2.338×10^{-27}	0.21
19	NP_249117.1	<i>mexB</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux transporter MexB		1.165×10^{-26}	18.91
20	NP_253474.1		PA4786 probable short-chain dehydrogenase		4.820×10^{-26}	27.32
21	NP_253664.1	<i>arvI</i>	2-ketoarginine decarboxylase, ArvI		7.288×10^{-26}	27.23
22	–		DNA mismatch repair protein MutL (pseudogene)		1.131×10^{-25}	11.29
23	NP_249234.1		PA0543		1.178×10^{-24}	1414
24	NP_249929.1		PA1238 probable outer membrane component of multidrug efflux pump		3.144×10^{-24}	6.14
25	NP_250447.1	<i>cysH</i>	3'-phosphoadenosine-5'-phosphosulfate reductase	L2P	5.950×10^{-23}	12.11
26	NP_252760.1		PA4071		8.727×10^{-23}	11.69
27	–		hypothetical protein		6.694×10^{-22}	11.20
28	NP_253053.1	<i>iciA</i>	inhibitor of chromosome initiation IciA	M1L	2.644×10^{-21}	11.68
29	NP_253724.1		PA5037		3.797×10^{-21}	16.65
30	NP_249976.1		PA1285 probable transcriptional regulator	D3V	4.551×10^{-21}	18.45
31	NP_253108.1	<i>ftsI</i>	penicillin-binding protein 3	R504C	1.290×10^{-20}	70.55
32	–		fused response regulator/phosphatase (pseudogene)		8.894×10^{-20}	58.28
33	–		transporter (pseudogene)		1.339×10^{-19}	1082
34	–		3-oxoacyl-ACP reductase (pseudogene)		1.426×10^{-19}	7.29
35	NP_249140.1		PA0449	W38R	5.887×10^{-19}	26.55
36	NP_249140.1		PA0449	G39S	5.887×10^{-19}	26.55
37	NP_250674.1	<i>exaC</i>	NAD+ dependent aldehyde dehydrogenase ExaC		2.493×10^{-18}	4.02
38	NP_251593.1	<i>cobJ</i>	precorrin-3 methylase CobJ	T4A	3.509×10^{-18}	4.60
39	NP_254125.1		PA5438 probable transcriptional regulator	272 deletion	6.325×10^{-17}	31.10

Rank	Accession	Gene name	Protein name	Mutation	p value	Odds ratio
40	NP_253015.1		PA4325	K28E	7.394×10^{-17}	NA
41	NP_064724.1	<i>gyrB</i>	DNA gyrase subunit B	S466F	2.944×10^{-16}	480.90
42	NP_253777.1	<i>vgrG5</i>	VgrG5	A242T	5.622×10^{-16}	6.06
43	NP_248891.1		PA0200	E64D	6.834×10^{-16}	4.01
44	NP_251237.1		PA2547 probable transcriptional regulator	E124D	1.049×10^{-16}	0.18
45	NP_252799.1	<i>ampC</i>	beta-lactamase precursor	V239A	2.917×10^{-15}	NA
46	—		cystine ABC transporter substrate-binding protein (pseudogene)		8.836×10^{-15}	NA
47	NP_248841.1		PA0151probable TonB-dependent receptor	E597Q	1.416×10^{-14}	0.12
48	NP_251742.1		PA3052	H18R	1.924×10^{-14}	8.59
49	NP_253604.1	<i>nadD2</i>	nicotinate mononucleotide adenylyltransferase NadD2	S7Y	2.039×10^{-14}	4.35
50	—		transporter		2.764×10^{-14}	0.28
51	NP_248708.1	<i>fnt</i>	methionyl-tRNA formyltransferase	I196V	3.410×10^{-14}	0.22
52	—		hypothetical protein		1.255×10^{-13}	0.28
53	NP_249116.1	<i>mexA</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux membrane fusion protein MexA precursor		2.070×10^{-13}	12.25
54	NP_253847.1		drug efflux transporter		2.767×10^{-13}	34.00
55	—		hypothetical protein		3.430×10^{-13}	0.24
56	NP_253820.1		PA5133		3.921×10^{-13}	4.74
57	NP_253669		PA4982 probable two-component sensor	N904S	4.612×10^{-13}	20.17
58	—		aldehyde dehydrogenase (pseudogene)		1.102×10^{-12}	12.00
59	NP_251831.1	<i>wbpM</i>	nucleotide sugar epimerase/dehydratase WbpM	V113P	1.296×10^{-12}	0.17
60	NP_249842.1	<i>imm2</i>	pyocin S2 immunity protein		1.308×10^{-12}	0.26
61	NP_254006.1	<i>radC</i>	DNA repair protein RadC	G73A	1.345×10^{-12}	0.06
62	NP_254006.1	<i>radC</i>	DNA repair protein RadC	D212G	1.345×10^{-12}	0.06
63	NP_254006.1	<i>radC</i>	DNA repair protein RadC	G213N	1.345×10^{-12}	0.06
64	NP_251813.1		PA3123	S37T	1.772×10^{-12}	0.28
65	NP_251232.1		PA2542	1130 insertion	3.018×10^{-12}	NA
66	—		hypothetical protein		3.890×10^{-12}	4.42

Rank	Accession	Gene name	Protein name	Mutation	p value	Odds ratio
67	NP_249718.1	<i>amaB</i>	delta1-Piperideine-6-carboxylate dehydrogenase	A253D	5.582×10^{-12}	4.98
68	NP_252709.1	<i>mpl</i>	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase		5.886×10^{-12}	17.06
69	NP_249004.1		L-cysteine transporter of ABC system YecS	164 insertion	7.611×10^{-12}	NA
70	NP_249415.1		PA0724 probable coat protein A of bacteriophage Pf1		8.949×10^{-12}	0.32
71	NP_248733.1		PA0043	W4R	1.034×10^{-11}	4.66
72	–		hypothetical protein		1.914×10^{-11}	0.10
73	NP_250922.1	<i>pslB</i>	PslB		2.495×10^{-11}	6.16
74	NP_249491.1		PA0800	H78R	2.584×10^{-11}	10.95
75	–		N-acetyltransferase GCN5		2.709×10^{-11}	0.36
76	–		hypothetical protein		2.842×10^{-11}	9.74
77	NP_251089.1	<i>pvdD</i>	pyoverdine synthetase D		3.156×10^{-11}	2.59
78	NP_253485.1		PA4797 probable transposase		5.499×10^{-11}	0.28
79	NP_251376.1	<i>pfeR</i>	two-component response regulator PfeR	V281A	1.101×10^{-10}	0.16
80	NP_249109.1		PA0418	T455R	1.769×10^{-10}	2.83
81	NP_250108.1		PA1417 probable decarboxylase	T262A	1.903×10^{-10}	3.06
82	NP_252773.1	<i>cupB3</i>	usher CupB3		2.964×10^{-10}	8.44
83	NP_250640.1	<i>rbsK</i>	ribokinase	L129M	3.806×10^{-10}	0.07
84	NP_249286.1	<i>lptD</i>	LPS-assembly protein LptD	N734T	3.890×10^{-10}	0.31
85	NP_253663.1	<i>aruH</i>	Arginine:Pyruvate Transaminase, AruH	R84Q	4.820×10^{-10}	0.21
86	NP_251980.1	<i>tle1</i>	Tle1	N131S	5.027×10^{-10}	11.03
87	–		hypothetical protein		6.531×10^{-10}	2.98
88	–		UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase (pseudogene)		6.735×10^{-10}	27.74
89	–		hypothetical protein		8.905×10^{-10}	2.89
90	NP_248921.1	<i>pcaB</i>	3-carboxy-cis,cis-muconate cycloisomerase	A298T	1.002×10^{-9}	0.09
91	NP_251114.1	<i>pvdL</i>	PvdL	V3938I	1.077×10^{-9}	3.15
92	–		hypothetical protein		1.098×10^{-9}	0.36

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>p value</i>	<i>Odds ratio</i>
93	NP_253337.1	<i>cupE1</i>	Pilin subunit CupE1		1.302×10^{-9}	NA
94	NP_249019.1	<i>aaaA</i>	arginine-specific autotransporter of <i>Pseudomonas aeruginosa</i> , AaaA	H516R	1.363×10^{-9}	0.36
95	NP_251146		PA2456		1.464×10^{-9}	0.27
96	NP_250319.1		PA1628 probable 3-hydroxyacyl-CoA dehydrogenase	R48Q	1.534×10^{-9}	0.15
97	NP_251184.1	<i>mexF</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux transporter MexF		1.720×10^{-9}	7.39
98	NP_250030.1	<i>aatP</i>	AatP		2.013×10^{-9}	NA
99	NP_251435.1		PA2745 probable hydrolase	L88G	2.641×10^{-9}	10.29
100	NP_250246.1	<i>ccoP2</i>	Cytochrome c oxidase, cbb3-type, CcoP subunit		3.364×10^{-9}	10.46
101	NP_251439.1	<i>endA</i>	DNA-specific endonuclease I	D151I	5.611×10^{-9}	21.44
102	NP_250442.1		PA1751	1 insertion	6.738×10^{-9}	2.82
103	NP_252454.1		PA3765	S62R	6.761×10^{-9}	0.27
104	NP_250687.1		PA1997 probable AMP-binding enzyme	A328T	9.248×10^{-9}	22.50

B.14. Dataset 4 treeWAS results

Table 31: Genes and SNPs identified with treeWAS from dataset 4, reduced dataset using features identified from random forest in datasets 1 and 2. Variants are ranked based on absolute simultaneous association score.

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Association score</i>	<i>p value</i>
1	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H3R	50.5	0
2	NP_250018.1		PA1327 probable protease		-50	0
3	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H12R	49.5	0
4	NP_249374.1	<i>hxcY</i>	HxcY		-49	0
5	NP_249865.1	<i>napA</i>	periplasmic nitrate reductase protein NapA		-46	0
6	NP_253053.1	<i>iciA</i>	inhibitor of chromosome initiation IciA	M1L	44	0
7	NP_248888.1	<i>tonB2</i>	TonB2		-44	0
8	NP_253957.1		PA5270	S5A	43	0
9	NP_064723.1	<i>recF</i>	RecF protein		-42	0

Rank	Accession	Gene name	Protein name	Mutation	Association score	p value
10	NP_250442.1		PA1751	1 insertion	-41	0
11	–		hypothetical protein		40	0
12	NP_250447.1	<i>cysH</i>	3'-phosphoadenosine-5'-phosphosulfate reductase	L2P	39	0
13	NP_248733.1		PA0043	W4R	38	0
14	NP_249538.1		diguanylate cyclase	D34G	36	0
15	NP_249491.1		PA0800	H78R	36	0
16	NP_253724.1		PA5037		-35	0
17	NP_250764.1		PA2074		33	0
18	NP_251980.1	<i>tle1</i>	Tle1	N131S	29	0
19	NP_253462.1		PA4774	X350E	-28.5	0
20	NP_251980.1	<i>tle1</i>	Tle1	I109F	28	0
21	NP_249976.1		PA1285 probable transcriptional regulator	D3V	27	0
22	NP_253781.1		PA5094 probable ATP-binding component of ABC transporter	X277Q	-27	0
23	NP_250105.1		PA1414		27	0
24	NP_249292.1	<i>agtR</i>	two-component response regulator, AgtR	1 deletion	-25	0
25	NP_249711.1		PA1020 probable acyl-CoA dehydrogenase	S8T	25	0
26	NP_251148.1		PA2458		-22	0
27	–		hypothetical protein		22	0
28	NP_251980.1	<i>tle1</i>	Tle1	I231V	21	0
29	NP_250876		PA2186		-21	0
30	NP_251089.1	<i>pvdD</i>	pyoverdine synthetase D		-21	0
31	–		hypothetical protein		-21	0
32	NP_250674.1	<i>exaC</i>	NAD+ dependent aldehyde dehydrogenase ExaC		-20.5	0
33	NP_251742.1		PA3052	H18R	20	0
34	NP_249140.1		PA0449	W38R	19	0
35	NP_249140.1		PA0449	G39S	19	0
36	–		hypothetical protein		19	0
37	–		hypothetical protein		18.5	0
38	–		hypothetical protein		18	0
39	–		hypothetical protein		-18	0
40	–		hypothetical protein		18	0
41	–		hypothetical protein		-18	0
42	NP_251435.1		PA2745 probable hydrolase	L88G	17	0
43	NP_252532		PA3843		17	0
44	–		non-ribosomal peptide synthetase (pseudogene)		17	0
45	NP_251593.1	<i>cobJ</i>	precorrin-3 methylase CobJ	T4A	-16	0

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Association score</i>	<i>p value</i>
46	NP_248961.1		PA0270		-16	0
47	–		hypothetical protein		-16	0
48	NP_248764.1	<i>ppkA</i>	serine/threonine protein kinase PpkA	L295M	-15	0
49	NP_249454.1	<i>mucA</i>	anti-sigma factor MucA		-15	0
50	–		tryptophan repressor binding protein		15	0
51	–		hypothetical protein		15	0
52	NP_249417.1		PA0726 hypothetical protein of bacteriophage Pf1		-15	0

B.15. Dataset 4 random forest results

Table 32: Genes and SNPs identified with RF from dataset 4, reduced dataset using features identified from random forest in datasets 1 and 2. Variants with importances greater than or equal to 0.002 are reported.

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Importance</i>
1	NP_250764.1		PA2074		0.268
2	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H3R	0.09
3	NP_248888.1	<i>tonB2</i>	TonB2		0.042
4	NP_254125.1		PA5438 probable transcriptional regulator	272 deletion	0.036
5	–		mutL (pseudogene)		0.027
6	NP_248708.1	<i>fnt</i>	methionyl-tRNA formyltransferase	I196V	0.022
7	NP_064723.1	<i>recF</i>	RecF protein		0.021
8	NP_250018.1		PA1327 probable protease		0.021
9	NP_249454.1	<i>mucA</i>	anti-sigma factor MucA		0.02
10	NP_250980.1	<i>gcd</i>	glucose dehydrogenase		0.018
11	NP_249004.1		L-cysteine transporter of ABC system YecS	164 insertion	0.015
12	–		tRNA-Ala		0.013
13	–		non-ribosomal peptide synthetase (pseudogene)		0.012
14	NP_251134.1	<i>glyA2</i>	serine hydroxymethyltransferase		0.011
15	–		DNA-binding response regulator (pseudogene)		0.01
16	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H12R	0.01
17	NP_253724.1		PA5037		0.009
18	NP_251249.1		PA2559	A161S	0.009
19	NP_252760.1		PA4071		0.008
20	–		hypothetical protein		0.008
21	NP_248783.1	<i>tse6</i>	Tse6	387 deletion	0.007

Rank	Accession	Gene name	Protein name	Mutation	Importance
22	NP_250319.1		PA1628 probable 3-hydroxyacyl-CoA dehydrogenase	R48Q	0.007
23	NP_249838.1		PA1147 probable amino acid permease	V396A	0.006
24	NP_251601.1		PA2911 probable TonB-dependent receptor	Y684D	0.005
25	–		16S ribosomal RNA		0.005
26	NP_249116.1	<i>mexA</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux membrane fusion protein MexA precursor		0.005
27	NP_249842.1	<i>imm2</i>	pyocin S2 immunity protein		0.005
28	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	K92R	0.004
29	NP_253663.1	<i>aruH</i>	Arginine:Pyruvate Transaminas, AruH	R84Q	0.004
30	–		hypothetical protein		0.004
31	NP_251114.1	<i>pvdL</i>	PvdL	V3938I	0.004
32	NP_064724.1	<i>gyrB</i>	DNA gyrase subunit B	S466F	0.004
33	NP_253604.1	<i>nadD2</i>	nicotinate mononucleotide adenylyltransferase NadD2	S7Y	0.004
34	NP_248937.1		PA0246 probable major facilitator superfamily (MFS) transporter	Y43F	0.004
35	NP_251435.1		PA2745 probable hydrolase	L88G	0.003
36	NP_250415.1	<i>pscK</i>	type III export protein PscK	E137D	0.003
37	NP_249140.1		PA0449	W38R	0.003
38	NP_249939.1	<i>aprF</i>	Alkaline protease secretion outer membrane protein AprF precursor	T82A	0.003
39	NP_253777.1	<i>vgrG5</i>	VgrG5	A242T	0.003
40	–		3-oxoacyl-ACP reductase (pseudogene)		0.003
41	NP_251093.1	<i>fpvG</i>	FpvG	K68T	0.003
42	NP_253820.1		PA5133		0.003
43	NP_254209.1	<i>pauA6</i>	Glutamylpolyamine synthetase	N276H	0.003
44	–		hypothetical protein		0.003
45	NP_252007.1		PA3317	S126N	0.003
46	NP_250809.1		alcohol dehydrogenase (Zn-dependent)		0.003
47	NP_249234.1		PA0543		0.003
48	NP_251973.1		PA3283	G26E	0.003
49	NP_249865.1	<i>napA</i>	periplasmic nitrate reductase protein NapA		0.003
50	NP_248783.1	<i>tse6</i>	Tse6	A392L	0.003
51	NP_253522.1		PA4835	A421E	0.003
52	NP_249381.1	<i>pdtA</i>	phosphate depletion regulated TPS partner A, PdtA		0.003
53	NP_253339.1	<i>cupE3</i>	Pilin subunit CupE3	N19S	0.003
54	NP_253191.1	<i>opdD</i>	Glycine-glutamate dipeptide porin OpdP	E30A	0.003

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Importance</i>
55	–		hypothetical protein		0.003
56	NP_252973.1	<i>recD</i>	exodeoxyribonuclease V alpha chain	A26V	0.003
57	NP_250470.1		assimilatory nitrate reductase	T549S	0.002
58	NP_248891.1		PA0200	E64D	0.002
59	–		hypothetical protein		0.002
60	NP_250687.1		PA1997 probable AMP-binding enzyme	A328T	0.002
61	NP_253485.1		PA4797 probable transposase		0.002
62	NP_252918.1	<i>pchD</i>	pyochelin biosynthesis protein PchD		0.002
63	NP_252773.1	<i>cupB3</i>	usher CupB3		0.002
64	–		hemagglutinin (pseudogene)		0.002
65	NP_250683.1		PA1993 probable major facilitator superfamily (MFS) transporter	G402V	0.002
66	NP_248921.1	<i>pcaB</i>	3-carboxy-cis,cis-muconate cycloisomerase	A298T	0.002
67	NP_252177.1	<i>tle5</i>	Tle5		0.002
68	NP_253179.1	<i>magD</i>	MagD	M1009V	0.002
69	NP_251838.1	<i>wbpI</i>	UDP-N-acetylglucosamine 2-epimerase WbpI		0.002
70	NP_251146		PA2456		0.002
71	NP_248997.1		PA0306 probable transcriptional regulator	R302C	0.002
72	NP_251980.1	<i>tle1</i>	Tle1	N131S	0.002
73	NP_250871.1		PA2181		0.002
74	NP_250674.1	<i>exaC</i>	NAD ⁺ dependent aldehyde dehydrogenase ExaC		0.002
75	–		hypothetical protein (pseudogene)		0.002
76	NP_253167.1	<i>cafA</i>	cytoplasmic axial filament protein	T416I	0.002
77	–		cystine ABC transporter substrate-binding protein (pseudogene)		0.002
78	NP_252799.1	<i>ampC</i>	beta-lactamase precursor	V239A	0.002
79	NP_249140.1		PA0449	G39S	0.002
80	NP_253576.1		PA4889 probable oxidoreductase	P350S	0.002
81	NP_249782.1	<i>fgtA</i>	flagellar glycosyl transferase, FgtA		0.002
82	–		phytanoyl-CoA dioxygenase		0.002
83	NP_252916.1	<i>pchE</i>	dihydroaeruginosic acid synthetase	D1001E	0.002
84	NP_250981.1		PA2291 probable glucose-sensitive porin		0.002
85	–		hypothetical protein		0.002
86	NP_253781.1		PA5094 probable ATP-binding component of ABC transporter	X277Q	0.002
87	NP_251519.1		PA2829	G15A	0.002
88	–		hypothetical protein		0.002
89	NP_253191.1	<i>opdD</i>	Glycine-glutamate dipeptide porin OpdP	N31T	0.002

B.16. Dataset 4 support vector machine results

Table 33: Genes and SNPs identified with SVM from dataset 4, reduced dataset using features identified from random forest in datasets 1 and 2. Top 100 variants with absolute hyperplane coefficients greater than 0.03 are reported.

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Hyperplane coefficient</i>
1	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H12R	0.9060
2	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	H3R	-0.6211
3	NP_254125.1		PA5438 probable transcriptional regulator	272 deletion	-0.5358
4	NP_251831.1	<i>wbpM</i>	nucleotide sugar epimerase/dehydratase WbpM	V113P	-0.5228
5	NP_248708.1	<i>fnt</i>	methionyl-tRNA formyltransferase	I196V	0.5130
6	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	K92R	-0.4968
7	NP_249976.1		PA1285 probable transcriptional regulator	D3V	0.4829
8	NP_253053.1	<i>iciA</i>	inhibitor of chromosome initiation IciA	M1L	0.4712
9	NP_250447.1	<i>cysH</i>	3'-phosphoadenosine-5'-phosphosulfate reductase	L2P	-0.4707
10	NP_248733.1		PA0043	W4R	-0.4622
11	NP_249939.1	<i>aprF</i>	Alkaline protease secretion outer membrane protein AprF precursor	T82A	0.4617
12	NP_250442.1		PA1751	1 insertion	0.4608
13	NP_251249.1		PA2559	A161S	0.4548
14	NP_250319.1		PA1628 probable 3-hydroxyacyl-CoA dehydrogenase	R48Q	0.4525
15	NP_249004.1		L-cysteine transporter of ABC system YecS	164 insertion	-0.4520
16	NP_251435.1		PA2745 probable hydrolase	L88G	0.4464
17	NP_250108.1		PA1417 probable decarboxylase	T262A	0.4444
18	NP_249286.1	<i>lptD</i>	LPS-assembly protein LptD	E812K	0.4416
19	NP_251993.1		PA3303 probable major facilitator superfamily (MFS) transporter	H262R	-0.4302
20	NP_250663.1	<i>pqqF</i>	pyrroloquinoline quinone biosynthesis protein F	C240G	0.4301
21	NP_250310.1		PA1619 probable transcriptional regulator	I14V	0.4205
22	NP_251980.1	<i>tle1</i>	Tle1	I109F	-0.4158
23	NP_251358		PA2668	5 insertion	-0.4119
24	NP_251232.1		PA2542	1130 insertion	-0.4111

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Hyperplane coefficient</i>
25	NP_251315.1		PA2625	V84I	-0.4084
26	NP_249533.1		PA0842 probable glycosyl transferase	K251R	-0.4048
27	NP_252886.1	<i>bfiS</i>	BfiS	E431A	0.4030
28	NP_249622.1	<i>pirA</i>	ferric enterobactin receptor PirA	A370T	-0.4014
29	NP_249286.1	<i>lptD</i>	LPS-assembly protein LptD	D593N	0.3995
30	NP_252466.1	<i>xseA</i>	exodeoxyribonuclease VII large subunit	L325Q	0.3975
31	NP_253191.1	<i>opdD</i>	Glycine-glutamate dipeptide porin OpdP	E30A	0.3882
32	NP_248937.1		PA0246 probable major facilitator superfamily (MFS) transporter	Y43F	-0.3803
33	NP_251716.1		PA3026	E464Q	0.3742
34	NP_251210.1	<i>czcA</i>	Resistance-Nodulation-Cell Division (RND) divalent metal cation efflux transporter CzcA	R595K	0.3720
35	NP_253957.1		PA5270	S5A	0.3671
36	NP_248783.1	<i>tse6</i>	Tse6	387 deletion	-0.3646
37	NP_248891.1		PA0200	E64D	0.3617
38	NP_253549.1		PA4862 probable ATP-binding component of ABC transporter	A174T	-0.3613
39	NP_251601.1		PA2911 probable TonB-dependent receptor	Y684D	-0.3572
40	NP_248783.1	<i>tse6</i>	Tse6	A392L	-0.3530
41	NP_253939.1		PA5252 probable ATP-binding component of ABC transporter	T225I	-0.3479
42	NP_253462.1		PA4774	X350E	0.3470
43	NP_252350.1		PA3660 probable sodium/hydrogen antiporter	A14T	-0.3449
44	NP_248969.1		PA0278	V111A	0.3422
45	NP_253191.1	<i>opdD</i>	Glycine-glutamate dipeptide porin OpdP	N31T	0.3377
46	NP_252017.1		PA3327 probable non-ribosomal peptide synthetase	E402K	-0.3365
47	NP_251604.1		PA2914 probable permease of ABC transporter	I255V	-0.3307
48	NP_251115.1	<i>pvdG</i>	PvdG	D178E	-0.3277
49	NP_251358		PA2668	R4L	0.3271
50	NP_249925.1		PA1234	N39D	-0.3233
51	NP_254006.1	<i>radC</i>	DNA repair protein RadC	D212G	-0.3174
52	NP_250687.1		PA1997 probable AMP-binding enzyme	A328T	0.3173
53	NP_251593.1	<i>cobJ</i>	precorrin-3 methylase CobJ	T4A	0.3170
54	NP_248764.1	<i>ppkA</i>	serine/threonine protein kinase PpkA	L295M	-0.3158
55	NP_253663.1	<i>aruH</i>	Arginine:Pyruvate Transaminas, AruH	R84Q	-0.3158

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Hyperplane coefficient</i>
56	NP_252007.1		PA3317	S126N	0.3148
57	NP_249290.1		PA0599	A20T	0.3140
58	NP_250415.1	<i>pscK</i>	type III export protein PscK	E137D	-0.3120
59	NP_253413.1	<i>cbrA</i>	two-component sensor CbrA	T16A	-0.3100
60	NP_250612.1		PA1922 probable TonB-dependent receptor	V419A	0.3100
61	NP_250774.1		PA2084 probable asparagine synthetase	V118A	0.3084
62	NP_251003		PA2313	H205O	0.3076
63	NP_250806.1		PA2116	T148A	0.3001
64	NP_250939.1	<i>bkdB</i>	branched-chain alpha-keto acid dehydrogenase (lipoamide component)	S321G	0.2972
65	NP_251749.1	<i>pelF</i>	PelF	N229S	0.2967
66	NP_251519.1		PA2829	G15A	0.2958
67	NP_250692.1		PA2002	V457G	0.2916
68	NP_251005.1		PA2315	V284I	-0.2892
69	NP_249124.1		PA0433	H116Q	-0.2890
70	NP_249633.1		PA0942 probable transcriptional regulator	D172A	0.2835
71	NP_254011.1	<i>sphR</i>	Sphingosine-responsive Regulator, SphR	D347E	-0.2818
72	NP_251124.1		PA2434	A145T	-0.2816
73	NP_251005.1		PA2315	L306V	-0.2811
74	NP_250470.1		assimilatory nitrate reductase	T549S	0.2801
75	NP_251358		PA2668	R4I	0.2779
76	NP_251157.1	<i>foxR</i>	Anti-sigma factor FoxR	A28G	0.2770
77	NP_252126.1		PA3436	H92R	-0.2762
78	NP_253777.1	<i>vgrG5</i>	VgrG5	A242T	0.2760
79	NP_253179.1	<i>magD</i>	MagD	M1009V	0.2736
80	NP_251602.1		PA2912 probable ATP-binding component of ABC transporter	S185G	0.2735
81	NP_249292.1	<i>agtR</i>	two-component response regulator, AgtR	1 deletion	-0.2729
82	NP_249158.1		PA0467	G140A	0.2711
83	NP_251114.1	<i>pvdL</i>	PvdL	V3938I	-0.2711
84	YP_008719755.1	<i>srfA</i>	SrfA	F87R	-0.2688
85	NP_253669		PA4982 probable two-component sensor	N904S	-0.2685
86	NP_250356.1	<i>fha2</i>	Fha2	G277S	-0.2681
87	NP_250705.1	<i>liuA</i>	putative isovaleryl-CoA dehydrogenase	A179P	-0.2678
88	NP_249019.1	<i>aaaA</i>	arginine-specific autotransporter of <i>Pseudomonas aeruginosa</i> , AaaA	H516R	-0.2677
89	NP_250023.1		PA1332	C48R	-0.2667
90	NP_250630.1		PA1940	T284A	0.2664
91	NP_252454.1		PA3765	S62R	0.2649
92	NP_252030.1		PA3340	S181N	0.2647

<i>Rank</i>	<i>Accession</i>	<i>Gene name</i>	<i>Protein name</i>	<i>Mutation</i>	<i>Hyperplane coefficient</i>
93	NP_253231.1	<i>lepA</i>	Pseudomonas aeruginosa-derived large extracellular protease, LepA	S550N	-0.2647
94	NP_251970.1	<i>oprO</i>	Pyrophosphate-specific outer membrane porin OprO precursor	F86Y	0.2638
95	NP_251813.1	<i>tle1</i>	PA3123	S37T	-0.2634
96	NP_251980.1		Tle1	N131S	0.2618
97	NP_249399.1		PA0708 probable transcriptional regulator	G181S	0.2617
98	NP_249104.1	<i>chpA</i>	component of chemotactic signal transduction system	V594L	0.2615
99	NP_249104.1	<i>chpA</i>	component of chemotactic signal transduction system	E825A	-0.2612
100	NP_252913.1		PA4223 probable ATP-binding component of ABC transporter	S484R	0.2602

C. ADDITIONAL TEST RESULTS

C.1. Results from random forest after condensing dataset 1 using 95% similarity profiles

Table 34: Importance of genes in random forest models before and after condensing the dataset using a 95% similarity threshold. If genes shared 95% of the same presence/absence profile with another gene(s), the genes were condensed into a single variable. The majority of genes condensed were either core genes or genes found in PA during a longitudinal study of one patient.

<i>Accession</i>	<i>Protein name</i>	<i>Importance</i>	<i>Original Importance</i>
NP_250764.1	PA2074	0.28	0.237
NP_249454.1	MucA	0.116	0.123
NP_253724.1	PA5037	0.061	0.041
NP_248888.1	TonB2	0.032	0.084
NP_251800.1	PA3110	0.022	0
NP_249415.1	probable coat protein A of bacteriophage Pf1	0.014	0.005
NP_251184.1	MexF	0.011	0.011
NP_252532	PA3843	0.011	0.022
NP_064723.1	RecF	0.01	0.029
	hypothetical protein (pseudogene)	0.009	0
	MutL (pseudogene)	0.008	0.009
	non-ribosomal peptide synthetase (pseudogene)	0.008	0.015
NP_249842.1	pyocin S2 immunity protein	0.007	0.004
	DNA-binding response regulator (pseudogene)	0.007	0.01

<i>Accession</i>	<i>Protein name</i>	<i>Importance</i>	<i>Original Importance</i>
NP_249929.1	probable outer membrane component of multidrug efflux pump	0.006	0.009
NP_250710.1	MexZ	0.005	0
	ffs	0.005	0
	3-oxoacyl-ACP reductase (pseudogene)	0.005	0.002
	hypothetical protein	0.005	0
	nan	0.005	0
NP_249381.1	phosphate depletion regulated TPS partner A, PdtA	0.004	0.008
NP_250809.1	alcohol dehydrogenase (Zn-dependent)	0.003	0.001
	nan	0.003	0
NP_251015.1	PA2325	0.003	0
	efflux transporter periplasmic adaptor subunit (pseudogene)	0.003	0.002
NP_253820.1	PA5133	0.003	0.008
NP_249673.1	PA0982	0.003	0
	nan	0.003	0.004
	ABC transporter ATP-binding protein (pseudogene)	0.003	0
NP_252709.1	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase	0.002	0.002
NP_248790.1	PA0100	0.002	0
	cystine ABC transporter substrate-binding protein (pseudogene)	0.002	0.003
	hypothetical protein (pseudogene)	0.002	0.001
NP_249865.1	periplasmic nitrate reductase protein NapA	0.002	0.003
NP_252177.1	Tle5	0.002	0.005
	transcriptional regulator	0.002	0.003
	glyA (pseudogene)	0.002	0.001
	hypothetical protein	0.002	0
	[]	0.002	0
	TetR family transcriptional regulator (pseudogene)	0.002	0.005
NP_250118	PA1427	0.002	0.006
	polyhydroxybutyrate depolymerase (pseudogene)	0.001	0
NP_249677.1	PA0986	0.001	0
	hypothetical protein	0.001	0
NP_252057.1	PA3367	0.001	0
	hypothetical protein	0.001	0

<i>Accession</i>	<i>Protein name</i>	<i>Importance</i>	<i>Original Importance</i>
NP_251876.1	Glucose/carbohydrate outer membrane porin OprB precursor	0.001	0
	emrB (pseudogene)	0.001	0
	[]	0.001	0
	hypothetical protein	0.001	0.001
NP_251028.1	probable binding protein component of ABC maltose/mannitol transporter subtype I-F CRISPR-associated endonuclease Cas1	0.001	0
	peptidase	0.001	0.003
	nan	0.001	0
	hypothetical protein	0.001	0
NP_251838.1	UDP-N-acetylglucosamine 2-epimerase WbpI	0.001	0.002
	hypothetical protein	0.001	0
	nan	0.001	0.004
	hydrolase (pseudogene)	0.001	0
	O-antigen biosynthesis protein	0.001	0
	[]	0.001	0
	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase (pseudogene)	0.001	0.002
NP_250420.1	PA1729	0.001	0
	nan	0.001	0
	hypothetical protein	0.001	0
	hypothetical protein	0.001	0
	hypothetical protein	0.001	0
	hypothetical protein	0.001	0
NP_250162.1	PA1471	0.001	0
	hypothetical protein similar to coat protein A of bacteriophage Pf1	0.001	0
NP_250203.1	secreted protein Hcp	0.001	0
	paraquat-inducible protein A	0.001	0
NP_251134.1	serine hydroxymethyltransferase	0.001	0.005
NP_250074.1	PA1383	0.001	0.005
	[]	0.001	0
NP_251508.1	aminoglycoside response regulator	0.001	0
NP_250059.1	PA1368	0.001	0.002
NP_251031.1	probable ATP-binding component of ABC maltose/mannitol transporter	0.001	0
	beta-lactamase	0.001	0

<i>Accession</i>	<i>Protein name</i>	<i>Importance</i>	<i>Original Importance</i>
NP_250018.1	probable protease	0.001	0.004
	[]	0.001	0
	[]	0.001	0
	pilus assembly protein FimV (pseudogene)	0.001	0
	nan	0.001	0
	nan	0.001	0
	nan	0.001	0
	hypothetical protein	0.001	0
	chemotaxis protein (pseudogene)	0.001	0
	hypothetical protein	0.001	0.001
NP_251618.1	PA2928	0.001	0
	hypothetical protein	0.001	0
	hypothetical protein	0.001	0
YP_008719781.1	PA4638	0.001	0
	nan	0.001	0
	hypothetical protein	0.001	0
NP_253389.1	PA4701	0.001	0
NP_253378.1	PA4689	0.001	0
	hypothetical protein	0.001	0
	nan	0.001	0.001
	nan	0.001	0
	acetyl-CoA acetyltransferase	0.001	0
	[]	0.001	0.001
NP_250908.1	PA2218	0.001	0
	hemagglutinin (pseudogene)	0.001	0
NP_248948.1	PA0257	0.001	0.006
	hypothetical protein	0.001	0
	hypothetical protein	0.001	0
	hypothetical protein	0.001	0
NP_249374.1	HxcY	0.001	0.004
	nan	0.001	0
	hypothetical protein	0.001	0
	cobyrinic acid a,c-diamide synthase	0.001	0
	5S ribosomal RNA	0.001	0
	hypothetical protein	0.001	0.003
	hypothetical protein	0.001	0
	hydrolase	0.001	0
	nan	0.001	0.002
	hypothetical protein	0.001	0
NP_249252.1	hypothetical protein	0.001	0
	lipoprotein	0.001	0.001
NP_250876	PA2186	0.001	0.002
NP_253664.1	2-ketoarginine decarboxylase, AruI	0.001	0.002
	hypothetical protein	0.001	0.002

<i>Accession</i>	<i>Protein name</i>	<i>Importance</i>	<i>Original Importance</i>
NP_249316.1	PA0625	0.001	0
NP_249188.1	PA0497	0.001	0
	hypothetical protein (pseudogene)	0.001	0
	sigma-54-dependent Fis family transcriptional regulator (pseudogene)	0.001	0
NP_248987.1	Glutamylpolyamine synthetase	0.001	0
NP_252906.1	probable pyridoxamine 5'-phosphate oxidase	0.001	0
	nan	0.001	0
NP_252903.1	phenazine biosynthesis protein PhzE	0.001	0
	ABC transporter substrate-binding protein	0.001	0
NP_252711.1	hydrazine dehydrogenase, HdhA	0.001	0
NP_249603.1	PA0912	0.001	0
	DNA-binding protein	0.001	0.003
	[]	0.001	0
	hypothetical protein	0.001	0
NP_251011.1	gluconokinase	0.001	0
	hypothetical protein	0.001	0
	cysteine synthase (pseudogene)	0.001	0
	hypothetical protein	0.001	0
NP_250922.1	PslB	0.001	0.001
	carboxylate-amine ligase (pseudogene)	0.001	0.001
	tRNA-Ile	0.001	0
	nan	0.001	0
	hypothetical protein	0.001	0
	acetyltransferase	0.001	0
	ABC transporter ATP-binding protein (pseudogene)	0.001	0