Whole genome of *Klebsiella aerogenes* PX01 isolated from San Jacinto River sediment west of Baytown, Texas reveals the presence of multiple antibiotic resistance determinants and mobile genetic elements

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1. Direct link to deposited data

https://www.ncbi.nlm.nih.gov/nuccore/NJBB00000000.

2. Experimental design, materials and methods

Polluted sediment was collected from the banks of the San Jacinto River along the north face of Burnet Bay in Baytown, Texas as part of an Environmental Sampling Research Module with the goal of tracking pesticide-degrading activity and isolating putative bacterial degraders across the Houston-metropolitan area [1]. Selective media was prepared for screening purposes: Carbon Selective Media (CSM) which has a composition of 2 mM NTA, 0.8 mM MgSO₄·7H₂O, 0.17 mM Ca(NO₃)₂, 0.018 mM FeSO₄·7H₂O, 20% v/v Phosphate Buffer. 5 mL of CSM media was aliquoted into culture tubes with 100 μg/mL ethyl paraoxon as a screening agent. These tubes were prepared fresh each week for each new subculture set for a period of five weeks. The culture was then diluted into minimal media with glycerol added as a supplementary carbon source and plated onto an agar plate with 100 μg/mL ethyl paraoxon. A resulting tan colored bacterium was isolated from the agar plate and shipped to Genewiz (South Plainfield, NJ), where library construction and whole genome sequencing of the bacterium was performed as described below.

Samples were visually inspected upon receipt and genomic DNA was extracted from bacterial colonies using the PureLink Genomic DNA extraction kit as per manufacturer’s protocols. The resulting genomic DNA was quantified using both the Nanodrop and the Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). A total of 50–60 ng of each sample was run on a 0.6% agarose gel to check for quality. The Illumina Nextera XT library preparation, clustering, and sequencing reagents were used throughout the process following the manufacturer’s recommendations (Illumina, San Diego, CA, USA). DNA libraries were analyzed on the Agilent TapeStation (Agilent Technologies, Palo Alto, CA, USA) and quantified using the Qubit 2.0 Fluorometer.
The DNA libraries were quantified by real time PCR (Applied Biosystems, Carlsbad, CA, USA), and multiplexed in equal molar mass. DNA libraries were multiplexed and loaded on an Illumina MiSeq instrument according to manufacturer’s instructions (Illumina, San Diego, CA, USA). Sequencing was performed using a 2 × 250 paired-end (PE) configuration. Image analysis and base calling were conducted by the MiSeq Control Software (MCS) on the MiSeq instrument (Table 1). Sequence reads were checked for quality using Fastqc and filtered using BBTools with minimum Phred score of 20. Paired-end reads were assembled into contigs with the Spades 3.10.1 program. Preliminary reference based annotation using PATRIC web resources was carried out to identify conserved pathways. Final de novo annotation performed through the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html) and the Rapid Annotation System Technology (RAST) server identified the unknown bacterium as Klebsiella aerogenes [6,7].

2.1. Data description

Klebsiella species are Gram-negative encapsulated bacteria commonly found in mammalian gastrointestinal tracts. While typically benign, some species do possess the capacity to act as opportunistic human pathogens. Klebsiella aerogenes (formerly Enterobacter aerogenes) is a motile, non-spore forming, bacterium that has emerged as a multidrug-resistant (MDR) threat and is often included as part of the ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter sp.) group of human pathogens, bacteria that are the source of most clinical infections worldwide [8]. Hospital outbreaks due to K. aerogenes is often related to the acquisition of novel antibiotic resistance determinants through mobile genetic elements as well as constitutive β-lactamase overexpression [9].

The genome of Klebsiella aerogenes PX01 includes a circular bacterial chromosome with a GC content of 65.09%, consists of 5,224,354 bp and a single plasmid of 38,598 bp. Combined, the total genome of PX01 contains approximately 5265 gene sequences, 5123 coding sequences including 41 rRNAs and 89 tRNAs. An overview of genome subsystem features (Fig. 1) shows that the greatest number of identified genes were foremost allocated to subsystems for general cell survival and metabolism including carbohydrate and protein metabolism as well as amino acid and vitamin synthesis and degradation. Secondary subsystems with over 100+ genes identified through RAST are principally survival and stress response oriented and include both cell wall and capsule production as well as antibiotic resistance and virulence.

| Assembly statistics | Illumina MiSeq (2 * 250) paired end |
|---------------------|----------------------------------|
| Platform            | Total raw reads                  |
|                     | 3,622,205                        |
|                     | Total filtered reads             |
|                     | 3,476,896                        |
| Genome size (bp)    | 5,224,354 (chromosome) 38,598 (plasmid) |
| Number of contigs   | 97 (chromosome) 6 (plasmid)     |
| Average coverage    | 240.65 ×                        |
| Annotation statistics | GC content                      |
|                     | 55.09% (chromosome) 51.34% (plasmid) |
| Total genes         | 5265                             |
| Coding genes        | 5,123                            |
| tRNAs               | 41                               |
| tRNAs               | 89                               |

![Fig. 1. Subsystem category distribution of major protein coding genes of Enterobacter aerogenes strain PX01 as annotated by the RAST annotation server. The bar chart on the left shows the subsystem coverage in percentage (blue bar corresponds to percentage of proteins included). The bar chart to the right shows the distribution of the 27 most abundant subsystem categories.](image-url)
analysis (Table 2) of the PX01 strain reveals a microorganism that is well suited for opportunistic pathogenicity through a combination of multidrug efflux, β-lactamase activity and antibiotic inactivation enzymes, many of which are also associated with clinical strains of both *K. pneumoniae* and *E. coli* [10]. In addition, this strain harbors a minimum of 46 prophage related sequences located on the bacterial chromosome suggesting a possible vehicle for lateral transfer to and from this bacterial strain.

**Conflict of interest**

The authors declare no conflict of interest.

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Table 2

| Category                                         | Hits | Notable genes involved in antibiotic resistance                      |
|--------------------------------------------------|------|---------------------------------------------------------------------|
| Determinant of sulfonamide resistance             | 1    | leuO                                                                |
| Determinant of fluoroquinolone resistance         | 1    | mfd                                                                |
| Antibiotic target protection protein              | 1    | mfd                                                                |
| Determinant of fosfomycin resistance              | 2    | FoxA5, UhpT                                                         |
| Determinant of ionized resistance                 | 1    | katG                                                               |
| Antibiotic resistant gene variant or mutant       | 2    | katG, UhpT                                                         |
| Determinant of aminoglycoside resistance          | 1    | kdpE                                                               |
| Determinant of beta-lactam resistance             | 1    | CMY-108                                                            |
| Antibiotic inactivation enzyme                    | 2    | CMY-108, FoxA                                                      |
| Protein modulating permeability to antibiotic     | 2    | marA, ramA                                                        |
| Gene altering cell wall charge                    | 4    | armA, PmrE, PmrC, PmrF                                            |
| Determinant of polymyxin resistance               | 4    | armA, PmrE, PmrC, PmrF                                            |
| Gene conferring antibiotic resistance via molecular bypass | 1    | bacA                                                              |
| Determinant of resistance to peptide antibiotics  | 1    | bacA                                                              |
| Protein(s) and two-component regulatory system modulating antibiotic efflux | 13   | adelC, adelD, CRP, marA, H-NS, kdpE, ramA, cpxA, baex, baex, emrE, leuO, robA |
| Efflux pump complex or subunit conferring antibiotic resistance | 43   | mdIC, robA, emrA, mdtH, mdtK, acrI, oqxA, mdtA, oqxB, mdtD, acrA, acrB, rosfB, rsoA, emrB, K. pneumoniae acrA, acrD, mdtM, patA, mceB, mceB, tolC, acrI, mdtG, mdtI, cpxA, mdtA, yojI, mdoA, emrD, baex, baex, norB, crp, hmrM, emrE, marA, adelC, adelD, ramA, macA, H-NS |