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De Novo Whole Genome Sequencing Data of *Pseudomonas aeruginosa* ATCC10145, an Opportunistic Pathogen

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen that is commonly found in nosocomial infections. *P. aeruginosa* is one of the most frequent model bacterial species, with the genomes of hundreds of strains of this species have been sequenced to date. This study aimed to analyze the whole genome sequence of *P. aeruginosa* ATCC® 10145™. The whole genome of the *P. aeruginosa* ATCC® 10145™was sequenced by shotgun sequencing using Genomic DNA Mini Kit, genome Analyzer$_{IIX}$ with 100-bp paired-end reads and genomic DNA extracted using the MasterPure complete DNA and RNA purification kit and complete genome sequence analysis was done. The genome of the *P. aeruginosa* ATCC® 10145™ was sequenced on the IlluminaMiseq platform. The raw sequenced reads were assessed for quality using FastQC v.0.11.5 and filtered for low quality reads and adapter regions using Trimmomatic v.0.36. The *de novo* genome assembly was made with CLC Genomics Workbench 5.1 and annotated using Prokaryotic Genome Annotation Pipeline (PGAP) v4.10. Here, we report the whole genome sequence of *P. aeruginosa* ATCC® 10145™ strain. All filtered and assembled genomic data sequences have been submitted to National Centre for Biotechnology Information (NCBI) and can be located at DDBJ/ENA/GenBank under the accession of VAOQ00000000 (UniSZA) and BioProject number PRJNA533327 and ID: 533327). The high-quality *P. aeruginosa* ATCC® 10145™ genome sequence provides a reference for further research including investigation of horizontal gene transfer or comparative genomics.

***Keywords*:** Whole genome sequencing, *Pseudomonas aeruginosa*, Genome assembly, Virulence factor

Introduction

P. aeruginosa is a gram-negative bacteria that can be found in soil, marshes, and coastal marine settings, as well as on plant and animal tissues.[1,2] It forms biofilms on wet surfaces such as those of rocks and soil.[3-6] Biofilm-grown bacteria can be up to 1000 times more resistant to antibiotics than their planktonic equivalents,[6-10] while biofilm cells themselves employ a variety of mechanisms to resist the action of antimicrobial agents.[6-8]*Pseudomonas aeruginosa* is ubiquitous in the environment inhabiting diverse natural environments.[11, 12] *P. aeruginosa* potentially possesses four chemotaxis systems, at least one of which contributes to its ability to form biofilms.[8, 13-16] This species is an opportunistic human pathogen and one of the WHO's priority pathogens list for research and development of antibiotics.[13, 15] *P. aeruginosa* is characterized by its intrinsic resistance to several antimicrobial agents in addition to the acquisition of the resistance genes by horizontal transfer and it has one of the largest bacterial genome.[17] *P. aeruginosa* was previously described as the second most common organism responsible for infections acquired in intensive care units (ICUs).[18] The demographic structure of *P. aeruginosa* is believed to be the consensus panmictic-epidemic,[18] i.e. phenotypically clonal with frequent recombination that creates new strains with unique genetic characteristics. The emergence of successful epidemiological copies.

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In addition, the clinical isolates are indistinguishable from ecological isolates; There is no specific clones related to a particular habitat.[18] Molecular epidemiological investigations have become essential for effective infection surveillance and outbreak detection.[18] To date, more than 500 genomes of *P. aeruginosa* strains have been sequenced, which has helped to reveal the evolutional history and adaptation mechanisms of the species.[19-21] The recent sequencing of the genome of the standard laboratory strain of *P. aeruginosa* PAO1 has laid the groundwork for more extensive studies of genomic variation in environmental isolates of this bacterium. Here we report the sequencing of the genome of *P. aeruginosa* ATCC® 10145™. The sequence is of interest because of the insights it provides into the role of this bacterium as a pathogen, and because it offers new information on the relationship between genome size, genetic complexity and ecological versatility in bacteria.[20]

Materials and Methods

Strain and growth conditions

P. aeruginosa ATCC®10145™ was purchased from the American Type Culture Collection (ATCC). *P. aeruginosa* was stored at –80°C in nutrient broth (NB) medium (Oxoid, UK) with 20% (v/v) glycerol. Prior to the assay, *P. aeruginosa* strain was sub-cultured from the frozen stock preparation onto nutrient agar (NA) plates (Oxoid, UK). The plates were incubated at 37°C for 24 hours. Pure liquid cultures of *P. aeruginosa* were maintained in nutrient broth.[5,9, 22, 23]

Genome sequencing and assembly

A single colony of *P. aeruginosa* was aseptically picked from the fresh culture from a nutrient agar plate using a sterile wire loop swabs and then were suspended in 2 mL of nutrient broth using a shaker incubator (30ºC, 200 rpm) for 24 hours. 1 mL of cell suspension was

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transferred to a 1.5 mL centrifuge tube and was centrifuged at 13,000 rpm for 1 min for harvesting cells. The cells were used for chromosomal DNA extraction using Genomic DNA Mini Kit (RBC, Taiwan). Genome sequencing of *P. aeruginosa* ATCC® 10145™ was performed using the Genome Analyzer$_{IIX}$ (Illumina, San Diego, CA, USA) with 100-bp paired-end reads. Genomic DNA extracted using the MasterPure complete DNA and RNA purification kit (catalog number MC85200; Lucigen) was used for both Illumina and Nanopore sequencing. DNA was fragmented and libraries prepared for Illumina sequencing using the NEXTflex Rapid DNA-seq kit (catalog number NOVA-5149-02; Bioo Scientific). Illumina paired-end reads (1,158,342, 2 × 150-bp reads) were generated on the MiSeq platform (Illumina, San Diego, CA, USA) using the IlluminaMiSeq reagent kit v2 (catalog number MS-102-2002). The paired-end reads were trimmed and *de novo* assembled using CLC Genomics Workbench 5.1. (Quiagen). Default parameters were used for all software unless otherwise specified. The draft genome was annotated using Blast2GO 2.5.0 (BioBam) and subsequently validated using Rapid Annotation Subsystems Technology (RAST) and the Bacterial Annotation System (BASys). The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v4.10. [5, 24-26]

Results and Discussion

This annotation is for the public version of the genome, whereas the other annotation workflows, as stated in Table 1, were employed. The *de novo* assembly yielded 5,684 proteins with an aggregate length of 5,639,658 bp, a G+C content of 61.18%, and containing 5,645 protein coding genes (CDS). The contig N_{50} was 1,197 bp and contig L_{50} was 1,590 bp. Of the CDSs, 3.5% were associated with the cell wall and capsule, 3.6% are associated with virulence, disease, and defense mechanisms, and 2.7% are related to the stress response, which contributes to adaptation in the host and survivability. Meanwhile, 3.14% are related to membrane transport, part of which is believed to contribute to the antibiotic resistance mechanism of the strain.

Table 1: Key features of *P. aeruginosa* ATCC® 10145™genome (PRJNA533327) (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA533327)

Features	Genome
Total length (DNA, total number of bases)	5,639,658 bp
DNA G + C content (%)	61.18
Genes (total)	5,662
CDSs (total)	5,660
Genes (coding)	5,645
CDSs (with protein)	5,645
Genes (RNA)	2
rRNAs	1, 1 (16S, 23S)
partial rRNAs	1, 1 (16S, 23S)
tRNAs	0
ncRNAs	0
Pseudo Genes (total)	15
CDSs (without protein)	15
Pseudo Genes (ambiguous residues)	0 of 15
Pseudo Genes (frameshifted)	10 of 15
Pseudo Genes (incomplete)	4 of 15
Pseudo Genes (internal stop)	1 of 15
CRISPR Arrays	5

P. aeruginosa ATCC® 10145™ genome assembly resulted in a single circular chromosome of 5,639,658 bp. A phylogenetic comparison of *P. aeruginosa* ATCC® 10145™ genome with other *P. aeruginosa* (PAO1161), *P. aeruginosa* (PAO1) and *P. aeruginosa* (PAO1-UW) genomes available in the NCBI database, identified a mucoid isolate from a patient with cystic fibrosis[27] as a strain with most similar genome. In the global analysis, *P. aeruginosa* ATCC® 10145™ localized close to the *P. aeruginosa* PAO1161, PAO1, and PAO1-UW containing branches, in agreement with its origin.[15, 27] We hypothesize that *P. aeruginosa* high genome size and genetic complexity reflect evolutionary adaptations that allow it to survive in a variety of ecological niches. The full genome sequence of *P. aeruginosa* gives several insights about the basis of this adaptability. *P. aeruginosa* has a diverse ability to transport, digest, and develop on organic chemicals, as well as various iron-siderophore absorption systems and an improved ability to export compounds (such as enzymes and antibiotics) via a variety of protein secretion and RND efflux systems. *Pseudomonas aeruginosa* may have four chemotaxis systems, at least one of which contributes to its ability to build biofilms.[15, 28] Thus, this organism can readily move to more favorable conditions or consolidate and 'dig in' for persistent colonization of a particular microenvironment. Consistent with its increased genetic complexity, *P. aeruginosa* has the greatest percentage of genes devoted to command-and-control systems (for example, environmental sensors and transcriptional regulators) observed in the bacterial genome. These regulatory genes presumably modulate the diverse genetic and biochemical capabilities of this bacterium in changing environmental conditions. *P. aeruginosa* infections are particularly difficult to treat because of intrinsic resistance to antibiotics. It would appear that, during evolving the functional diversity required to compete with other microorganisms in a variety of environments, it developed mechanisms for resisting naturally occurring antimicrobial compounds. The efflux systems we identified could contribute to this intrinsic resistance. Antimicrobial effects could potentially be reduced by altering the expression of drug targets, enzymatic modifiers, transport systems, and compensatory pathways. Indeed, *P. aeruginosa's* extremely large regulatory capabilities may allow for more flexibility for adaptive drug resistance via gene regulation than occurs in other bacteria with smaller genomes. Furthermore, given its ability to metabolize a wide range of organic substrates, *P. aeruginosa* may have a greater potential for enzymatic modification and degradative drug resistance mechanisms than previously assumed. As a result, *P. aeruginosa's* metabolic variety, transport skills, and regulatory adaptability, which allow it to thrive and compete with other microbes, all likely contribute to its high inherent resistance to antibiotics. The full genome sequence and encoded mechanisms give a wealth of information for the discovery and exploitation of new antibiotic targets, as well as promise for the development of more effective ways to treat life-threatening *P. aeruginosa* opportunistic infections in humans.[20] The epidemiology of nosocomial *P. aeruginosa* infection is complex because this pathogen is ubiquitous in the environment. Since *P. aeruginosa* is able to survive on wet surfaces such as ponds, pond traps, pipes, and hydrotherapy equipment, several hospital outbreaks have been associated with these specific reservoirs. This assumption was confirmed by results of whole genome sequencing showing that environmental isolates were highly similar to patients isolates.[18]

Conclusion

The purpose of this study was to characterize the whole genome sequence analysis of the *P. aeruginosa* ATCC® 10145™ strain. The whole genome sequencing method was utilized to gain a better understanding of the entire DNA sequences of the *P. aeruginosa* ATCC® 10145™ strain. Our findings point to a reasonably straightforward model for genetic diversity in *P. aeruginosa* ATCC® 10145TM's 5,639,658 bp genome. More research is needed to gain a better knowledge of the genetic features of *P. aeruginosa* ATCC® 10145™. This could eventually result in educated and proper community knowledge about preventing the spread and continued emergence of such deadly infections.

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Table 2: Sequence accession numbers and directory links

Species	Directory/Data	Accession number	Links
P. aeruginosa ATCC 10145	BioProject	PRJNA533327	https://www.ncbi.nlm.nih.gov/bioproject/533327
	BioSample	SAMN11457101	https://www.ncbi.nlm.nih.gov/biosample/SAMN11457101
	Raw sequence (SRA)	SRR12680080	https://trace.ncbi.nlm.nih.gov/Traces/sra/?run=SRR12680080
			https://www.ncbi.nlm.nih.gov/sra?linkname=bioproject_sra_all&from_uid=533327
	Assembled genome	ASM589314v1	https://www.ncbi.nlm.nih.gov/assembly?LinkName=bioproject_assembly_all&from_uid=533327

Data availability

This whole-genome draft sequence project has been deposited at DDBJ/ENA/GenBank under accession no. VAOQ00000000. The version of the genome described in this paper is the first version (GenBank accession no. VAOQ00000000 under BioProject number PRJNA533327 and ID: 533327). Unassembled reads are also available from the NCBI Sequence Read Archive (SRA) under the accession number SRR12680080 (Table 2).

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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