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Virulence genome analysis of *Pseudomonas aeruginosa* VRFPA10 recovered from patient with scleritis

Nandagopal Murugan [a,c], Jambulingam Malathi [a,*], Vetrivel Umashankar [b], Hajib Narahari Rao Madhavan [a]

[a] Dept of Microbiology, L & T Microbiology Research Centre, Vision Research Foundation, Sankara Nethralaya, Chennai, Tamil Nadu 6000 06, India
[b] Centre for Bioinformatics, Vision Research Foundation, Sankara Nethralaya, Chennai, Tamil Nadu 6000 06, India
[c] Scholar, School of Chemical & Biotechnology, SASTRA University, Thanjavur, Tamil Nadu 613401, India

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ABSTRACT

Infectious keratitis is a major cause of blindness, next to cataract and majority of cases are mainly caused by gram negative bacterium *Pseudomonas aeruginosa (P. aeruginosa)*. In this study, we investigated a *P. aeruginosa* VRFPA10 genome which exhibited susceptibility to commonly used drugs *in vitro* but the patient had poor prognosis due to its hyper virulent nature. Genomic analysis of VRFPA10 deciphered multiple virulence factors and *P.aeruginosa* Genomic Islands (PAGIs) VRFPA10 genome which correlated with hyper virulence nature of the organism. The genome sequence has been deposited in DDBJ/EMBL/GenBank under the accession numbers LFMZ01000001-LFMZ01000044.

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Specifications	
Organism/cell line/tissue	*Pseudomonas aeruginosa*
Strain	VRFPA10
Sex	N/A
Sequence or Array type	ION PGM
Data format	Analyzed
Experimental factors	Genomic DNA extracted from pure bacterial culture isolated from the Human scleral scraping
Experimental features	Draft genome sequence of *P. aeruginosa* VRFPA02, assembly and annotation
Consent	Approved
Sample Source Location	Vision Research Foundation, Sankara Nethralaya Eye Hospital Chennai, Tamil Nadu, India (13° 5″ 0.1212″ N, 80° 16″ 13.818″ E)

1. Direct link to deposited data

https://www.ncbi.nlm.nih.gov/nuccore/LFMZ00000000.1

2. Background

P.aeruginosa is the predominant gram negative bacterium often associated with ocular infections such as keratitis and scleritis. Scleritis is a severe painful condition caused by inflammatory process in the sclera, which may involve cornea, adjacent episclera and underlying uvea may turn into blindness condition [1]. In the current study, we investigated a 38 years old male patient with history of injury caused by foreign body and he was initially diagnosed and treated for perforated corneal ulcer. But, the patient subsequently developed into scleritis condition, despite appropriate medical management.

3. Materials and methods

The specimen was collected by an ophthalmologist as per standard method [2]. In brief, edge of the ulcer was firmly scraped using Bard Parker blade No. 15' after removal of debris or discharge in the vicinity. Several scrapings were collected and used in a sequence to inoculate culture media. Wherein, "C" curve on blood agar, MacConkey agar and inoculated on Brain Heart Infusion Broth (BHIB) initially. Upon confirmation of *P.aeruginosa* by biochemical methods, it was subcultured on Mueller Hinton agar plates to assess pigment production and was incubated at 37°C for 24 h.

After 24 h colonies greenish pigmented colonies morphologically resembling *P. aeruginosa* had grown in all the culture plates and identification was using a combination of colonial morphology with bluish

* Corresponding author at: Dept of Microbiology, L & T Microbiology Research Centre, Old no 18, College Road, Vision Research Foundation, Sankara Nethralaya, Chennai 600 006, Tamil Nadu, India.
E-mail address: drjm@snmail.org (J. Malathi).

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green pigmentation on MHA plate, non lactose fermenting colonies in Macconckey agar, presence of motility, positive reaction for oxidase, catalase, simmon's citrate medium, nitrate reduction and mannitol sugar test and negative reaction for urease, indole, Methyl Red, Vogues Prosker test, sucrose, lactose and maltose sugar tests were observed [3, 4].

Further the organism was genotypically confirmed up to species level using 16s ribosomal RNA gene based sequencing result against blast tool available at NCBI database revealed 99% homology to all the existing *P.aeruginosa* strains in the database inclusive of our previously reported strains VRFPA01-VRFPA09 [5–10], hence the strain isolated from scleritis was designated as *P.aeruginosa* VRFPA10. Irrespective of the fact that *P. aeruginosa* VRFPA10 was phenotypically susceptible but the patient finally underwent Therapeutic Penetrating Keratoplasty (TPK). Whole genome study was undertaken by utilizing Ion Torrent (PGM) sequencer with 400-bp read chemistry (Life Technologies) accordance with manufacturer's instructions. In brief, genomic DNA from VRFPA10 was isolated from the overnight cultures with DNeasy miniprep kit (Qiagen, Hilden, Germany) and the sequencing protocol was followed as per previous study [5–10].

4. Genomic analysis

It is to be noted that there are no or scanty study available on Virulence factor and molecular mechanism of pathogenesis in *P.aeruginosa* mediated scleritis [11–14]. Henceforth, we undertook this study to analyze VRFPA10 whole genome to unveil the genomic nature of virulence mechanism and drug resistance genes which may be involved in drug resistance in *in vivo* condition but may show susceptibility in *in vitro* tests.

Data of 56× coverage was produced after initial quality analysis and reference based assembly with *P.aeruginosa* VRFPA04 (CP008739.2) yielded 44 contigs with 7,728,786 bp (7.7 Mb genome size). The VRFPA10 genome was published in NCBI under the accession number LFMZ00000000.1. The genes were annotated by NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP, http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html). The genomic feature of VRFPA10 constitutes of 6431 genes and 5252 protein Coding Sequences (CDS). A total of 154 RNA encoding genes were identified, inclusive of 80 tRNA CDS, 73 rRNA CDS and 1 non-coding RNA encoding CDS. Multiple Locus Sequence Typing (MLST) analysis using Resfinder confirmed that VRFPA10 belongs to Sequence Type (ST)-313 reported by our group previously in VRFPA07 strain isolated from rectal swab of a different patient [6].

Complete genomic analysis was carried out using CLC Genomics Workbench v 6.5.1. All available betalactamase, aminoglycosidase, Sul, CatB, TetG, dfrB, fosA and other resistant gene databases were downloaded from Resfinder [14] and it was installed in CLC Genomics Workbench as a complete database. Complete drug resistant database based blast analysis against VRFPA10 genome detected blaPA0, blaOXA-50, CatB7, FosA and OqxB (olaquindox resistant gene) [15]. Among the genes mentioned above, blaPA0 and blaOXA-50 were integrated into the chromosomal genome of all available *P. aeruginosa* genome which confers resistance to I and II generation cephalosporins and penicillin group of drugs. Whereas, CatB7, FosA and OqxB gene confers resistance to Chloramphenicol, Fosfomycin and olaquindox drugs respectively [16]. All the features were described in Table. 1

Blast analysis against various available virulence databases from NCBI unveiled the presence of Type 3 Secretion system (T3SS) cytotoxin encoding exoenzymes such as Exo-U, Exo-T, Exo-Y gene, Elastase B and ExotoxinA encoding genes among VRFPA10 genome [17]. These Exotoxin A and Elastase B are known to have an effect on ocular tissues, which may may have facilitated to adjacent episcleral invasion leading to scleritis condition. Furthermore, *P.aeruginosa* Genomic Islands (PAGI) otherwise called Region of Genomic Islands (RGPs) were identified among VRFPA10 genome which constituted PAGI-1,2,3,4 and 5, whereas no or partial trace of PAGI-6,7,8,9,10 was found scattered among VRFPA10 genome. This RGPs could have been obtained by Horizontal Gene Transfer (HGT) or phage infected genomes from various bacterial and viral sources that may be involved in inducing the pathogenicity of this VRFPA10 genome. These findings on VRFPA10 genome will aid in drug discovery against corneal virulence factor which may be helpful in treating the patient with corneal ulcers in addition to empirical drug regimens used in routine medical management.

Table 1
Genomic Features of *P.aeruginosa* VRFPA04.

Features	VRFPA10
Specimen	Scleral scraping
NCBI accession no	LFMZ01000000
Genome size	7,728,786 bp
No of contigs	44
No of proteins	5252
No of genes	6431
CRISPR arrays	0
Ribosomal RNA	73
t RNA	80
Noncoding RNA	1
Pseudo genes	501
Frameshifted genes	555
Genome coverage	56×
Reference guided assembly	CP008739.2
NCBI Accession WGS ID	LFMZ01000001:LFMZ01000044
MLST Type	ST-313
Beta lactamases	blaTem,
Gene	Ctx-M-15
Total no of phages	ND
Aminoglycoside genes	aph(3″)IIb
Fosfomycin	fosA
Phenicol	CatB7
Tetracycline	ND
Trimethoprime	ND
Genomic Islands	PAGI-1,2,9 (partial)
Integron	ND
Pathogenic island	ND

ND - Not detected.

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