Whole Genome Sequencing and Genome Annotation of PGPR ‘Exiguobacterium sp. TNDT2’ Isolated from Dates Palm Tree Rhizospheric Soil

Thennarasu Sugumar¹, Punithavathi Srinivasan², B. Muthukumar³, E. Natarajan⁴

ABSTRACT

Background: Dates palm is one of the most economically important plant mainly cultivated in Northern Africa, Middle East and South Asia. In India, Dates palm is the largest importer of date fruit. In India, Dates palm are cultivated majorly in Gujarat and Rajasthan. Dates farmers facing several problem in India due to lack of scientific resources. Plant growth promoting rhizobacteria (PGPR) are naturally associated with plants and it improves plant growth and yield by providing growth supplements, increasing tolerance to stressful conditions and providing resistance to fungal/bacterial diseases. We have isolated a PGPR belonging to Exiguobacterium species TNDT2 from Indian dates palm Phoenix dactylifera, in Dindigul region, Tamilnadu, India. The organism’s genome was sequenced and identified several potential plant growth promoting (PGP) genes.

Methods: The organisms genome was sequenced using Whole genome shotgun sequencing method in Illumina platform. Sequences are analysed using various bioinformatics tools and assembled using Velvet assembler. Contigs are annotated using RAST server and deposited in NCBI.

Result: The isolated strain revealed various genetic determinants required for plant growth promotion. This study presents the first report of Exiguobacterium TNDT2 genome from Dates tree rhizosphere. Whole genome analysis and genome annotation reveals that, its genome consist of a 2,891,840 bp chromosome encoding over 3062 proteins, with a 51.63% GC content. Strain TNDT2 encodes a wide repertoire of proteins for plant growth promotion, heavy metal detoxification (cadmium, arsenic, mercury, copper and tellurite), Multi-drug resistance and stress resistance (Heat, cold and salt). Based on this study, Exiguobacterium sp. TNDT2 can be recognized as an important organism with a potential to be incorporated into agricultural practice of Date palm.

Key words: Exiguobacterium sp. TNDT2, Exiguobacterium, Extremophiles, Heavy metal, PGPR, Rhizosphere.

INTRODUCTION

The Date Palm (Phoenix dactylifera) is an economically valuable plant grown in Asian and Africa arid and semi-arid zones. It has a wide range of essential nutrients with a lot of dietary potassium (Lunde 1978). In India, few farmers favor growing Date Palm due to challenges associated with producing higher yield. Limited microbial data available for Dates plant makes designing their growth conditions difficult (Marasco et al. 2012). Supplemeting plant with Plant Growth Promoting Rhizobacteria (PGPR) has been shown to increases yield by improving plant tolerance to stressful condition (Marasco et al. 2012), producing plant hormones (de Zelcourt et al. 2013, Garcia-Pichel et al. 2003, Barazani and Friedman 1999), promoting biotic and abiotic stress resistance (Ryu et al. 2013), promoting tolerance to water shortage (de Zelcourt et al. 2013), solubilizing inorganic phosphate (Schachtman et al. 1998), siderophore production (Tian et al. 2009, Sharma et al. 2003), producing organic acids (Ndung’u-Magiroi et al. 2012), protecting from bacterial and fungal diseases (urnkranz et al. 2009), ammonia production (Wani et al. 2007). Since Dates trees are cultivated in dry regions, PGPR should withstand all the stressful conditions like high temperature, salinity, water shortage and support the growth of the tree.

Exiguobacterium is a orange pigmented, facultative anaerobe, motile, Gram positive bacteria with variable morphologies, ranging from small rods to cocci (Collins et al. 1983). Member of Genus of this organism isolated from various locations including cold and hot conditions temperature ranging from -12°C to 55°C (Vishnivetskaya et al. 2003). Barazani (2003), producing organic acids (Ndung’u-Magiroi et al. 2012), protecting from bacterial and fungal diseases (urnkranz et al. 2009), ammonia production (Wani et al. 2007). Since Dates trees are cultivated in dry regions, PGPR should withstand all the stressful conditions like high temperature, salinity, water shortage and support the growth of the tree.

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and Kathariou 2005, Vishnivetskaya et al. 2007), slightly alkaline and marine environment (Vishnivetskaya et al. 2009), plant rhizosphere (Rodrigues et al. 2007), biofilms (Carneiro et al. 2012), fresh water (Raichand et al. 2012), brine shrimp (Lopez-Cortes et al. 2006), ice (Chaturvedi and Shivaji 2006) and permafrost (Vishnivetskaya et al. 2006). Most members of the genus Exiguobacterium are polyextremophiles (Vishnivetskaya et al. 2014). These organisms are used in bioremediation and agricultural application. The biotechnological applications are to reduce heavy metals arsenate (Castro-Severyn et al. 2017), mercury (Petrova et al. 2002), Chromium (Okeke 2008); high catalase activity to remove peroxides in bleaching industry (Takebe et al. 2007); to remove pesticide (Lopez et al. 2005); to neutralize highly alkaline industry waste water (Kumar et al. 2006); and plant growth promotion (Dastager et al. 2010).

This study presents the first genomic report of Indian Dates tree PGPR strain Exiguobacterium. In the present study we aim to understand genetic determinants of various plant growth promoting activity. We identified many heavy metal resistance, multidrug resistance and stress related genes from this Whole Genome Sequencing (WGS) study.

MATERIALS AND METHODS

Bacterial growth condition and DNA Extraction

Exiguobacterium sp. strain TNDT2 isolated from Dates tree rhizosphere soil from Dindigul of Tamilnadu region, India. Bacteria was grown in LB medium for 48 hrs at 28C. DNA extraction was performed using QIAmp DNA mini kit (Qiagen) following the manufacturer’s instruction. DNA quality and quantity was checked spectrophotometrically (OD260/280 ratio).

Genomic DNA library construction and sequencing

Genomic DNA library was prepared using Nextera XT Library preparation kit (Illumina, USA) following the manufacturer’s recommendation. The library sized 75 bases sequenced using NextSeq 500 sequencer (Illumina).

Sequence Quality Control, Assembly and Annotation

The sequences quality was checked using FastQC (Andrews 2010) and all other analysis were carried out using the tools available in GALAXY (Afgan et al. 2018). The GALAXY is loaded in Amazon Cloud System and maintained by UC Davis bioinformatics group, was used for the genomic sequence analysis. There are different tools in GALAXY for the analysis and assembly. Adopter trimming was carried out using Scythe(https://github.com/ucdavis-bioinformatics/scythe). Incorrectly called bases in 5’ and 3’ end regions negatively impact the assembly. Those bad quality bases and reads with <Q30 value are trimmed off in Sickle (Joshi and Fass 2011). Denovo sequence assembly of short sequences were done using Velvet tool (Zerbino and Birney 2008, Zerbino et al. 1990) for quality and identity. Total contigs were annotated on RAST (Aziz et al. 2008) server and the contigs are deposited in GenBank. CGView server used to create genome map and check homology with another rhizospheric Exiguobacterium sp. MH3 Genbank no. CP006866 (White et al. 2019, Tang et al. 2013, Grant and Stothard 2008). The secondary metabolite biosynthetic gene clusters were predicted using antiSMASH v5.1.2 (Blin et al. 2017).

RESULTS AND DISCUSSION

The sequencing reaction generated a total of 4753514 filtered paired-end reads with the average size 76 nucleotides, providing 124- fold coverage of the genome. Different hash lengths tried for the better sequence alignment in Velvet. Hash length 47 provided less number of contigs (66 numbers) with a N50 length of 1,31,572 bp and total length 2,891,840 bp sequences. The GC content of Exiguobacterium sp. TNDT2 is 51.63%. RAST identified 3062 coding sequences (CDS) and 345 subsystems (Fig 1) in strain TNDT2. The Whole Genome Shotgun project has been deposited at GenBank (Table 1) under the accession number QLVE01000000, Bioproject number PRJNA476830. In CGViewer contigs are mapped with the help of reference

![Fig 1: Genes connected to subsystems and their distribution in different categories. Subsystem Coverage: 39% in subsystem, 61% not in subsystem. Total number of subsystems 345.](image_url)
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genome strain MH3 and CDS location identified using BLAST in CGViewer (Fig 2). Using antiSMASH v5.1.2 we have identified terpene biosynthetic gene clusters.

Based on the RAST annotation we found that strain TNDT2 possess several genes (Table 2) encoding protein related to plant growth promotion (Auxin, Catalase, Esterase/Lipase, Siderophore biosynthesis, Antibiotic biosynthesis and Ammonia production). Siderophore related genes are Siderophore biosynthesis protein monoxygenase, HtA, HtB and Hemin uptake protein. The enzyme L-asparaginase involved in ammonia production. We found many genes for Siderophore “Petrobactin” biosynthesis. The gene products are Petrobactin ABC transporter ATP-binding protein, Petrobactin ABC transporter permease protein I and Petrobactin ABC transporter permease protein II. Petrobactin is a bis-catecholate, α-hydroxy acid siderophore (Barbeau et. al. 2002). Siderophores are low molecular weight compounds, which are specific ferric chelating agents and it can promote the mineral dissolution of insoluble phases (Shirvani and Nourbakhsh, 2010). The general mechanism of siderophore-promoted Fe dissolution happens by forming the Fe(III)-siderophore complex at the mineral surface and transferred to the surrounding soil and then available for the uptake of microbes and plants (Kraemer, 2004).

Strain TNDT2 is highly motile organism. It has flagellar genes FlgG, FlgF, FlgB, FlgC, FlgD, FlaA, Fls, Fls, MotA and MotB. It also has fimbrial assembly genes Pla, Plb and PilC. Fimbria may helps the organism to attach on the host and leads to biofilm formation. In the genome, there is a gene called veg which involves in the biofilm formation (Lei et al. 2013). We have also identified genes responsible for Chemotaxis (CheA, CheC, CheD and CheV).

Genetic analysis of this organism revealed that it can degrade many potentially dangerous heavy metals-Arsenic, mercury, zinc, Lead, cadmium, copper and tellurite. The proteins identified for the degradation are Arsenate reductase (EC 1.20.4.1); Arsenic, Lead, cadmium, zinc and mercury transporting ATPase (EC 3.6.3.5); Copper-translocating P-type ATPase (EC 3.6.3.4); Cobalt-zinc-cadmium resistance protein CzcA; Mercuric ion reductase (EC 1.16.1.1); Anion permease ArsB/NhaD-like; Cadmium-transporting ATPase (EC 3.6.3.3); Tellurite resistance protein; Camphor resistance CrcB protein and Quaternary ammonium compound-resistance protein SugE. Strain TNDT2 has high potential in treating industrial waste water and reducing heavy metal toxicity in agricultural field.

Table 1: GenBank Submission Details.

| Subject                  | Details                  |
|--------------------------|--------------------------|
| Organism name            | Exiguobacterium sp. TNDT2|
| BioSample Number         | SAMN09460123             |
| BioProject Number        | PRJNA476830              |
| GenBank assembly accession| GCA_003331145.1          |
| RefSeq assembly accession | GCF_003331145.1          |
| WGS Project              | QLVE01                   |
| Total Sequence Length    | 2,891,840                |
| Number of contigs        | 66                       |
| Coding sequences         | 3062                     |
| GC content               | 51.6                     |
| N50 Value                | 1,31,572 bp              |
| Longest Contig           | 304488                   |

Fig 2: Genome plot of the Strain TNDT2 using CGViewer. Circular genome map made using genome Exiguobacterium sp. strain MH3 (GenBank: CP006866) a rhizoshere bacteria of Lemna minor. Aligned Contigs are in purple, CDS in yellow.
Most of the members of the Genus Exiguobacterium are extremophiles (Vishnivetskaya et al. 2009). Genome analysis of Strain TNDT2 reveals that it posses many stress related genes for Cold Shock (CspC, CspD), Heat Shock protein 60 family, chaperon GroEL, Heat shock protein HtpX, Carbon starvation protein A, HtrA - prevent heat misfolding of protein, heat shock protein Hsp20, Phosphate starvation-inducible protein PhoH and Alkaline shock protein. We also found genes responsible for capsular biosynthesis CapA, Cap5F. It may help the organisms to withstand high salinity. Normally Exiguobacterium species are non-spore former (Chen et al. 2017, Collins et al. 1983), but we found many proteins related to spore formation - Spore protease, Spore coat protein F, Stage V sporulation protein required for dehydratation of the spore core and assembly of the coat (SpoVS) and Sporulation initiation phosphotransferase (Spo0F). BLASTX search for similar sequences of spore related genes showed that many other Exiguobacterium genome also has these genes. Gene SpoVs showed similarity to Exiguobacterium sp. Strains AB2, AT1b, S17, SH31, Exiguobacterium chiriqhucha RW-2, Exiguobacterium mexicanum and Exiguobacterium antarcticum B7 in BLASTX search. Since strain TNDT2 has many spore related proteins, it may be a spore former. Our strain also possess transposase IS30, IS200/IS605 families. The most ancient Exiguobacterium sp. strain 255-15 isolated from 2-3 million-year-old Siberian permafrost, has numerous putative transposase sequences, primarily of the IS200/IS605, IS30 and IS3 families (Tatiana et al. 2005). BLASTX of IS200/IS605 family of strain TNDT2 showed similarity to Exiguobacterium sp.NG55, Exiguobacterium sp. AM39-5BH, Exiguobacterium sibiricum and many other Exiguobacterium.

RAST analysis also identified CRISPR elements in our strain TNDT2. CRISPR sequences detect and destroy bacteriophage DNA during subsequent infection hence play a key role in antiviral defence system (Barrangou 2015). These sequences are not present in Exiguobacterium chiriqhucha str. N139 (Gutiérrez-Preciado et. al. 2017), Exiguobacterium arabatum W01(Cong M et al. 2017) genomes.

This strain is a multidrug resistant one. The genome poses resistant genes for drugs vancomycin, tetracycline, chloramphenicol, rifampin, Bacitracin, Acriflavin, Streptomycin, Penicillin and Methicillin. The related genes are Vancomycin B-type resistance protein VanW, rifampin ADP-ribosyl transferase, Tetracycline resistance protein, rAd chloramphenicol resistance, Streptomycin acetyltransferase, Multidrug resistance protein B, Bacitracin transport permease protein BCRB, Acriflavin resistance protein, tunicamycin resistance protein, Penicillin-binding protein and Methicillin resistance protein. Exiguobacterium sp. strain S3-2 (Jing Yang et. al. 2014) is identified in marine fish farms has seven plasmid borne antibiotic resistance genes responsible for 5 antibiotics resistance namely Tetracyclin, Chloromphenicol, Streptomycin, Erythromycin and Trimethoprimum. Genomic study of Exiguobacterium sp. AT1b/GX59 (Chen et. al. 2017) showed the presence of antimicrobial resistance genes, including tetracycline resistance genes, macrolide resistance genes, aminoglycoside resistance genes, phenicol resistance genes, cationic antimicrobial peptide, multidrug resistance efflux pumps (abcA and bmrA) and vancomycin resistance modules (vanY, vanW). Exiguobacterium chiriqhucha strain RW2 isolated from cold fresh water microbialite in Pavillion Lake possess vancomycin and tetracyclin antibiotic resistance genes and testing revealed

### TABLE 2: GENETIC PROPERTIES OF EXIGUOBACTERIUM SP. TNDT2.

| FUNCTION                        | GENES/PROTEINS/PRODUCTS                                                                 |
|--------------------------------|-----------------------------------------------------------------------------------------|
| PLANT GROWTH PROMOTION         | CATALASE, LIPASE, AUXIN, SIDEROPHORE, PHOSPHATASE, HEAVY METAL DEGRADATION, AMMONIA PRODUCTION |
| HEAVY METAL DEGRADATION        | ARSENIC, MERCURY, CADMIUM, TELLURITE, LEAD, COPPER, COBALT, ZINC                         |
| MULTI-DRUG RESISTANCE          | VANCOMYCIN, TETRACYCLINE, CHLORAMPHENICOL, REFAMPIN, BACITRACIN, ACRIFLAVIN, STREPTOTHRICIN, PENICILLIN |
| STRESS-RESPONSE                | COLD SHOCK PROTEIN, HEAT SHOCK PROTEIN, OSMATIC STRESS, OXIDATIVE STRESS                |
| FLAGELLAR AND FIMBRIAL GENES   | FLGG, FLGF, FLGB, FLGC, FLGD, FLAA, FLIS, FLHS, PILA, PILB, PILC                          |
| INDUSTRIAL USEFUL ENZYMES      | ALPHA-AMYLASE (EC 3.2.1.1), FRUCTOKINASE (EC 2.7.1.4), NEOPULLULANASE (EC 3.2.1.135), PULLULANASE (EC 3.2.1.41), ESTERASE/ LIPASE, CATALASE (EC 1.11.1.6), AMIDASE, SERINE ALKALINE PROTEASE (SUBLISIN E), L.ASPARAGINASE (E.C.3.5.1.1), CARBOXYLSTERASE (EC 3.1.1.1) AND ENOLASE (EC 4.2.1.11) |
| SPORE FORMATION                | SPORE PROTEASE, SPORE COAT PROTEIN F, STAGE V SPORULATION PROTEIN REQUIRED FOR DEHYDRATATION OF THE SPORE CORE AND ASSEMBLY OF THE COAT (SPOVS) AND SPORULATION INITIATION PHOSPHOTRANSFERASE (SPO0F) |
| CAPSULE FORMATION              | CAPSULAR BIOSYNTHESIS CAPA, CAPSF                                                      |
it is sensitive to both antibiotics and resistance to sulfisoxazole (White et al. 2019).

Other industrial important enzymes producing genes in strain TNDT2 are Alpha-amylase (EC 3.2.1.1), Fructokinase (EC 2.7.1.4), Neopullulanase (EC 3.2.1.135), Pullulanase (EC 3.2.1.41), Esterase/Lipase, Catalase (EC 1.11.1.6), amidase, serine alkaline protease (subtilisin E), L-asparaginase (EC.3.5.1.1), Carboxylesterase (EC.3.1.1.1) and Enolase (EC 4.2.1.11). We also identified some other enzymes Phytoene desaturase, neurosporene or lycopene producing / 4,4'- diaplycopenone oxidase and Neurosporene desaturase. These enzymes involves in carotenoid neurosporene or lycopene biosynthesis. This may be responsible for colonies orange pigmentation. The carotenoid, neurosporene is an antioxidant for decades and a powerful antioxidant (Ciriminna et al. 2016).

CONCLUSION

The above results suggest that *Exiguobacterium* strain TNDT2 can be adopted to wide environments like high/moderate temperature, high salt and alkaline conditions. Most of the Dates tree grown in hot areas, this organism can be easily adopted to those environment. Since it possess many genes responsible for plant growth promotion auxin, catalase, esterase/lipase, heavy metal degradation, siderophore biosynthesis, antibiotic biosynthesis and ammonia production; it can be used as biofertilizers to increase the yield of Dates tree. This organism can be an excellent candidate for bio remediation application since we predicted many heavy metal degrading genes (Kumar et al. 2006, Castro-Severyn et al. 2017). Carotenoid production ability of this strain may have application in natural pigment biosynthesis.

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