Genomic sequence analysis of a plant-associated \textit{Photobacterium halotolerans} MELD1: from marine to terrestrial environment?

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**Abstract**

Mercury impacts the function and development of the central nervous system in both humans and wildlife by being a potent neurotoxin. Microbial bioremediation is an important means of remediation of mercury-contaminated soil. The rhizospheric \textit{Photobacterium halotolerans} strain MELD1 was isolated from mercury and dioxin contaminated site from Tainan, Taiwan. It has been shown to reduce Hg\textsuperscript{2+} to Hg\textsuperscript{0}. The 4,758,027 bp genome of \textit{P. halotolerans} MELD1 has a G + C content of 50.88 % and contains 4198 protein-coding and 106 RNA genes. Genomic analysis revealed the presence of a number of interesting gene cluster that maybe involved in heavy metal resistance, rhizosphere competence and colonization of the host plant.

**Keywords:** Mercury, Mer operon, Glycine-Betaine, ROS, Rhizosphere, Heavy metals, \textit{Photobacterium halotolerans}

**Abbreviations:** ROS, Reactive oxygen species

**Introduction**

Species of the \textit{Photobacterium} genus are Gram-negative bacteria belonging to the family of \textit{Vibrionaceae} [1] and has been known to be marine bacteria either pathogenic [2] or symbiotic to marine life [3]. \textit{Photobacterium halotolerans} was first reported by Rivas et al. [4] which was isolated from saline lake located in Mallorca, Spain.

In our previous study we isolated \textit{Photobacterium halotolerans} MELD1, a plant growth promoting gamma-proteobacterium that was isolated from the root of \textit{Phragmites communis} Trin. Ohwi [5], a large perennial grass found in wetlands throughout temperate and tropical regions of the world. The key feature of MELD1 was found to be the presence of \textit{mer} operon, consisting of mercury reductase gene (\textit{merA}) that helped in the conversion of Hg\textsuperscript{2+} to Hg\textsuperscript{0} [6]. It was noted that MELD1 was resistant to mercuric chloride concentration up to a concentration of 33 \(\mu\)g/ml. In the present study we summarize the genome classification of \textit{P. halotolerans} MELD1 along with its annotation for rhizosphere competence, plant growth promoting and heavy metal resistant genes.

**Organism information**

**Classification and features**

The genes encoding for the 16S rRNA were amplified by PCR using two universal primers, E8F and U1510R followed by BLAST against NCBI 16S rRNA sequences database. The 16S rRNA sequences of MELD1 and closely related strains were aligned by ClustalW and minimized with BioEdit (Tom Hall, Ibis Biosciences, Carlsbad, CA). The phylogenetic tree was generated by One Click phylogeny analysis on Methodes et Algorithmes pour la Bio-informatique Lirmm website [7] and exported by TreeGraph2 [8] (Fig. 1). Strain MELD1 demonstrated 99 % similarity to the \textit{Photobacterium halotolerans} MACL01T as compared to other \textit{Photobacterium} strains. Classification and general features of \textit{P. halotolerans} MELD1 are shown in Table 1.

Strain MELD1 is a Gram-negative bacterium, rod-shaped and motile by means of polar flagella. They are usually 2–4 \(\mu\)m in diameter (Fig. 2). \textit{P. halotolerans} MELD1 could
grow at 6 % NaCl as compared to P. halotolerans MACL01T which can grow at 8 % NaCl [4]. MELD1 was shown to utilize glucose, sucrose, maltose and α-D-Lactose as the sole carbon source.

Genome sequencing information

Genome project history
The genome project and the sequence were deposited in National Centre of Biotechnology Information [9]. MELD1 genome project consists of 62 contigs with a size of 4,758,037 bp, covering more than 97 % of the genome. A summary of the project information is showed in Table 2.

Growth conditions and genomic DNA preparation

P. halotolerans MELD1 was grown in Luria-Bertani medium under aerobic conditions at 28°C [4]. The genomic DNA was extracted by WelPrep DNA kit (Welgene Biotech, Cat No.D001). The size, purity and DNA concentration was measured by running pulse field gel electrophoresis, ratio of absorbance values at OD 260/280 in the range 1.8 ~ 2.0, and quantity ratio by Qubit versus NanoDrop over 0.7.

Genome sequence and assembly

DNA was sequenced using Illumina Solexa technology. Ten microgram of total DNA was sonicated by Misonix 3000 sonicator to the size ranging from 400 to 500 bp.

The genome size was estimated prior to assembly using Bioanalyzer DNA 1000 chip (Agilent Technologies). One microgram sonicated DNA was end-repaired, A-tailed and adaptor-ligated following the Illumina Trueseq DNA preparation protocol.

Genome annotation

ConDeTri [10] was implemented to trim or remove the reads according to the quality score and the cleaned and filtered nuclear reads were assembled de novo using Abyss [11]. The gene functions were annotated using NCBI Prokaryotic Genome Annotation Pipeline, which uses has automatic annotation pipeline that combines ab initio gene prediction algorithms with homology based methods.

Genome properties

MELD1 genome contained 62 contigs with a size of 4,758,037 bp. The G + C content was 50.90 % (Fig. 3 and Table 3). Of the total 4382 genes, 4176 are protein-coding genes and 105 are RNA genes. The classification of genes based on COG functions is shown in Table 4.

Insights from the genome sequence

Photobacterium halotolerans MELD1 has been isolated from the rhizosphere of Phragmites communis Trin., a plant found growing in mercury and dioxin contaminated land. In our previous study, we had demonstrated...
the presence of mer operon, which helped in the conversion of Hg$^{2+}$ to Hg$^0$. The Mer operon of MELD1 was compared to the most similar Gram-negative bacteria in the NCBI database. It was observed the genes merR, merT, merF, merP and merA had varying degree of similarity compared to Vibrio shilloni, Vibrio harveyi [12] and Shewanella frigidimarina [13] as shown in Fig. 4.

Since P. halotolerans MELD1 was isolated from a plant growing in heavy metal environment, the bacteria had genes responsible for it to be a rhizosphere or endophytic bacteria and genes responsible for heavy metal resistance. MELD1 encodes genes responsible for rhizosphere competence like Siderophore, Chemotaxis, Quorum sensing, Glycine-Betaine, Tyrosine recombinase.

Many bacteria acquire iron from the environment by secreting small iron-binding molecules called siderophores. Bacteria have developed several mechanisms to compete for iron, an important element required for their growth. Siderophores are known to have an antagonistic effect by depriving iron from other microorganisms [14]. The presence of an effective iron uptake system can therefore contribute to protect the host plant against phytopathogens. Acquisition of iron is an important trait for rhizosphere competition [15]. Similar to other Shigella spp [16], P. halotolerans MELD1 is able to synthesize the siderophore aerobactin, it also encodes ferric siderophore transport proteins. Plant growth-promoting genes like nitrate reductase, narL, ntrC and phosphate transporters (pst and pho) are found to be in the MELD1 genome. Analysis of GC content of MELD1 genome (51 %) portrays that the gene that appear to have a GC content close to that of MELD1 genome, could have been integrated into MELD1 genome through evolution by the process of horizontal gene transfer (Additional file 1) [17–19].

Analysis of genome revealed that MELD1 has a number of gene reported to play a role in osmotolerance like glycine-betaine and ectoine. The genome analysis of MELD1 revealed genes involved in glycine betaine synthesis that help MELD1 to maintain osmotic balance in hyper saline environment. It was observed that MELD1 was able to grow at a salt concentration of up to 6 %. It carries an ectABC cluster responsible for synthesis and accumulation of ectoine (Additional file 2). Since we isolated MELD1 from a heavy metal contaminated environment, we identified genes responsible for heavy

| Classification   | Domain Bacteria | TAS [32] |
|------------------|----------------|----------|
| Phylum           | Proteobacteria | TAS [33] |
| Class            | Gammaproteobacteria | TAS [34] |
| Order            | Vibrionales    | TAS [35] |
| Family           | Vibrionaceae   | TAS [1]  |
| Genus            | Photobacterium | TAS [36] |
| Species          | Photobacterium halotolerans \[6\] | TAS [4] |
| Type strain      | MELD1          | TAS [6]  |
| Gram stain       | Negative       | TAS [4]  |
| Cell shape       | Rod shaped     | TAS [4]  |
| Motility         | Not reported   | NAS      |
| Sporulation      | Not reported   | NAS      |
| Temperature range| 4–37 °C        | TAS [4]  |
| Optimum temperature | 28 °C       | TAS [6]  |
| pH range; Optimum| 5–8.5, 7.4 IDA | TAS [4]  |
| Carbon source    | Glucose, Sucrose, L-arabinose | TAS [4] |

**Table 1** Classification and general features of *Photobacterium halotolerans* MELD1 [31]

![Fig. 2](https://example.com)
metal resistance to such as mercury, arsenic, copper, and tellurium as well gene’s responsible for antibiotic resistance and antimicrobial compound like phenazine (Additional file 3).

Plants utilize a variety of defense mechanisms against various pathogens, including the production of ROS, hydrogen peroxide [20, 21]. Prior to root colonization, MELD1 has to survive in an oxidative rhizosphere environment. The genome contains a number of genes that can play a role in detoxification of reactive oxygen species commonly found in bacteria’s growing in toxic environments. It includes peroxidase, superoxide dismutase, alkyl hydroperoxidase, hydroperoxidase DNA repair protein and universal stress protein. The MELD1 chromosome encodes two superoxide dismutases: SodA, an Mn superoxide dismutase, and SodB, a Fe superoxide dismutase. Acriflavine resistance protein B is another stress resistant gene induced upon by plant colonization, but it’s not triggered by oxidative stress. The product of this gene encodes a component of the AcrAB-TolC efflux pump that is important in toxic waste removal in bacteria and their expression increased during stress conditions [22, 23] (Additional file 4).
Adhesion to the root in endophytic and rhizobacteria is mediated by cell surface structures such as polysaccharides, pili and adhesion [24]. It also carries a cluster of chemotaxis genes cheY, cheW, cheA, cheR and cheX (Additional file 5) and a cluster containing flg and fli genes responsible for flagella biosynthesis and motility (Additional file 6). It was also seen to possess the gene xerD, a site recombinase critical for the plant growth promoting rhizobacteria Psuedomonas fluorescens F113 to be an effective rhizosphere colonizer [25]. Quorum-sensing regulation gene in several strains of Azospirillum lipoferum [26] modulates functions related to rhizosphere competence and adaptation, such as siderophore synthesis, pectinase activity and indole acetic acid production [27]. MELD1 has quorum-sensing-regulatory genes like luxR and luxI, encodes AI-2 which is implicated in the regulation of biofilm formation and motility [28]. Some other genes involved for root adhesion including Hemaagglutinin [29, 30] are seemed to be responsible for the plant-microbe interaction as well as the twitching motility were observed in the MELD1 genome (Additional file 7).

**Conclusions**

The 4.7 Mb draft genome of Photobacterium halotolerans MELD1, a strain having mercury reductase activity has been deposited at NCBI under the accession number JWYV00000000. The version described in this study is the first version, JWYV01000000. MELD1 also contained a cluster of gene's responsible for heavy metal resistance, heavy metal efflux pumps, antimicrobial compounds, stress resistant, motility, and plant growth promoting genes, which all prove that they can function as a

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**Table 3** Genomic statistics for Photobacterium halotolerans MELD1

| Attribute                  | Value     | % of Total |
|----------------------------|-----------|------------|
| Genome Size (bp)           | 4,758,027 | 100.00     |
| G + C content (bp)         | 2,420,749 | 50.88      |
| DNA coding (bp)            | 4,054,779 | 85.22      |
| Number of scaffolds        | 57        |            |
| Total genes                | 4382      | 100.00     |
| RNA genes                  | 106       | 2.43       |
| Pseudogenes                | 65        | 1.49       |
| Protein-coding genes       | 4198      | 96.09      |
| Genes assigned to COGs     | 3509      | 80.32      |
| Genes assigned Pfam domain | 3650      | 83.54      |
| Genes with signal peptides | 407       | 9.32       |
| Genes with transmembrane helices | 1000 | 22.89 |
| CRISPR repeats             | 2         |            |

*The total is based on either the size of the genome in base pairs or the total number of protein coding genes*

**Table 4** Number of genes associated with the general COG functional categories

| Code | Value | % age | Description                                           |
|------|-------|-------|------------------------------------------------------|
| J    | 282   | 7.09  | Translation, ribosomal structure and biogenesis       |
| A    | 1     | 0.03  | RNA processing and modification                       |
| K    | 310   | 7.79  | Transcription                                         |
| L    | 138   | 3.47  | Replication, recombination and repair                 |
| B    | 1     | 0.03  | Chromatin structure and dynamics                      |
| D    | 49    | 1.23  | Cell cycle control, mitosis and meiosis               |
| Y    | 0     | 0     | Nuclear structure                                     |
| V    | 101   | 2.54  | Defense mechanisms                                    |
| T    | 241   | 6.06  | Signal transduction mechanisms                        |
| M    | 244   | 6.14  | Cell wall/membrane biogenesis                        |
| N    | 144   | 3.62  | Cell motility                                         |
| Z    | 0     | 0     | Cytoskeleton                                          |
| W    | 38    | 0.96  | Extracellular structures                             |
| U    | 93    | 2.34  | Intracellular trafficking and secretion               |
| O    | 185   | 4.65  | Posttranslational modification, protein turnover, chaperones |
| X    | 65    | 1.63  | Mobilome: prophages, transposons                      |
| C    | 208   | 5.23  | Energy production and conversion                      |
| G    | 239   | 6.01  | Carbohydrate transport and metabolism                 |
| E    | 326   | 8.2   | Amino acid transport and metabolism                   |
| F    | 94    | 2.36  | Nucleotide transport and metabolism                   |
| H    | 194   | 4.88  | Coenzyme transport and metabolism                     |
| I    | 139   | 3.5   | Lipid transport and metabolism                        |
| P    | 199   | 5     | Inorganic ion transport and metabolism                |
| Q    | 98    | 2.46  | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 332   | 8.35  | General function prediction only                      |
| S    | 256   | 6.44  | Function unknown                                      |
| -    | 0     | 0     | Not in COGs                                           |

*The total is based on the total number of protein coding genes in the genome*
rhizosphere or an endophytic bacteria in a toxic environment. The detailed genome announcement can give insight into the adaption of a marine dwelling bacterium as a terrestrial dwelling endophytic or rhizosphere bacterium and in future might aid in the bioremediation of mercury. Further more extensive research need to be done using molecular techniques to establish horizonal gene transfer in MELD1 with donor species.

Additional files

**Additional file 1:** Plant growth promoting genes. (DOCX 111 kb)  
**Additional file 2:** Genes responsible for osmotic stress resistance. (DOCX 50 kb)  
**Additional file 3:** Heavy metal and Antibiotic resistance genes. (DOCX 25 kb)  
**Additional file 4:** Genes responsible for detoxification. (DOCX 90 kb)  
**Additional file 5:** Genes responsible for rhizosphere competence. (DOCX 62 kb)  
**Additional file 6:** Gene responsible for motility. (DOCX 92 kb)  
**Additional file 7:** Genes responsible for secretion systems. (DOCX 69 kb)

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Author’s contributions
DCM and CCH initiated and supervised the study. DCM, SCL, GMM and KHC drafted the manuscript and annotated the genome. DCM performed electron microscopy. DCM and SCL worked on genome sequence and assembly. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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