

## Accepted Manuscript

Title: Complete genome of *Pseudomonas* sp. strain L10.10, a psychrotolerant biofertilizer that could promote plant growth

Author: Wah Seng See-Too Yan-Lue Lim Robson Ee Peter  
Convey David A. Pearce Wai-Fong Yin Kok Gan Chan



PII: S0168-1656(16)30063-3  
DOI: <http://dx.doi.org/doi:10.1016/j.jbiotec.2016.02.017>  
Reference: BIOTEC 7411

To appear in: *Journal of Biotechnology*

Received date: 30-1-2016  
Accepted date: 9-2-2016

Please cite this article as: See-Too, Wah Seng, Lim, Yan-Lue, Ee, Robson, Convey, Peter, Pearce, David A., Yin, Wai-Fong, Chan, Kok Gan, Complete genome of *Pseudomonas* sp. strain L10.10, a psychrotolerant biofertilizer that could promote plant growth. *Journal of Biotechnology* <http://dx.doi.org/10.1016/j.jbiotec.2016.02.017>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

## Complete genome of *Pseudomonas* sp. strain L10.10, a psychrotolerant biofertilizer that could promote plant growth

Wah Seng See-Too<sup>a,2b</sup>, Yan-Lue Lim<sup>a</sup>, Robson Ee<sup>a</sup>, Peter Convey<sup>b,c</sup>, David A. Pearce<sup>b,c,d</sup>, Wai-Fong Yin<sup>a</sup>, Kok Gan Chan<sup>a,e\*</sup>

<sup>a</sup> Division of Genetics and Molecular Biology, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

<sup>b</sup> National Antarctic Research Centre (NARC), Institute of Postgraduate Studies, University of Malaya, 50603 Kuala Lumpur, Malaysia

<sup>c</sup> British Antarctic Survey, NERC, High Cross, Madingley Road, Cambridge CB3 0ET, UK

<sup>d</sup> Faculty of Health and Life Sciences, University of Northumbria, Newcastle Upon Tyne NE1 8ST, UK

<sup>e</sup> UM Omics Centre, University of Malaya, Kuala Lumpur

\*Corresponding author: Institute of Biological Sciences (Division of Genetics and Molecular Biology), Faculty of Science, University of Malaya, 50603 Malaysia. Tel: +603-79675162. Email: kokgan@um.edu.my

### Highlights

- *Pseudomonas* sp. strain L10.10 is a psychrotolerant bacterium.
- This is the first complete genome of *Pseudomonas* sp..
- Various genes coding for potentially plant promoting properties were identified
- Functional annotation revealed an indole acetic acid (IAA)-producing attribute

### Abstract

*Pseudomonas* sp. strain L10.10 (=DSM 101070) is a psychrotolerant bacterium which was isolated from Lagoon Island, Antarctica. Analysis of its complete genome sequence indicates its possible role as a plant-growth promoting bacterium, including nitrogen-fixing ability and indole acetic acid (IAA)-producing trait, with additional suggestion of plant disease prevention attributes *via* hydrogen cyanide production.

**Keywords:** Plant disease control, nitrogen fixing, indole acetic acid, hydrogen cyanide, plant growth-promoting rhizobacteria

Plant growth-promoting rhizobacteria (PGPR) are of significant agricultural importance and biotechnological value due to their plant growth-promoting activity under stressful and nutrient limiting conditions (Nabti et al., 2010). *Pseudomonads* are well known PGPRs which are associated directly with plant growth promotion by the production of indole acetic acid (IAA) and involvement in nitrogen fixation activity (Zhao, 2010; Santi et al., 2013). In addition, *Pseudomonads* also confer indirect promotion of plant growth through production of hydrogen cyanide (HCN), which aids in prevention of plant diseases caused by phytopathogens (Schippers et al., 1990). Previously, we have isolated a psychrotolerant bacterium namely *Pseudomonas* sp. strain L10.10 from Antarctica. Psychrotolerant PGPRs are important in improving cold stress tolerance of important agricultural crops such as grapevine plantlets (Ait Barka et al., 2006). Hence, we investigated the genomic features of *Pseudomonas* sp. strain L10.10 in order to determine if this psychrotolerant strain has the relevant PGP genotype.

Genomic DNA of *Pseudomonas* sp. strain L10.10 was isolated from an overnight cell suspension culture using the MasterPure™ Gram positive DNA purification kit (Epicentre Technologies). Subsequently, the genomic DNA was constructed into a 20-kb SMRTbell template library. Pacific Biosciences (PacBio) RSII sequencing platform was used to perform whole genome sequencing using C4 chemistry in two single molecule real time (SMRT) cells (Ee et al., 2015, Lim et al., 2015). A total of 16,782 reads with a mean read length of 11,629 bp were generated. The reads were *de novo* assembled using hierarchical genome assembly process (HGAP) algorithm version 3 into a circular contig with an average genome coverage of 34.96-fold (Goh et al., 2015).

Genome annotation was then performed using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) version 2.10 and Rapid Annotation using Subsystem Technology (RAST) version 3.0 (Aziz et al., 2008, Overbeek et al., 2014, Brettin et al., 2015, Xia et al., 2015). The genome of *Pseudomonas* sp. strain L10.10 consists of a 5,172,488 bp circular chromosome

with G+C content of 58.2%. A total of 3514 protein coding genes were predicted along with 25 rRNA and 68 tRNA genes (Table 1).

Functional annotation of the genome revealed the presence of genes responsible for the two major pathways of bacterial ammonia assimilation, namely the glutamate dehydrogenase (GDH) pathway (glutamate dehydrogenase [AOC04\_00325]) and the glutamine synthetase (GS)-glutamate synthase (GOGAT) pathway (glutamine synthetase [AOC04\_04085] and glutamate synthase [AOC04\_01625]). In addition, a complete hydrogen cyanide synthase gene cluster [AOC04\_10125, AOC04\_10135, AOC04\_10140] whose products catalyze the synthesis of hydrogen cyanide were identified. Furthermore, we detected the presence of various genes which contribute to IAA biosynthesis, including Indole-3-glycerol phosphate synthase [AOC04\_22470], *N*-(5'-phosphoribosyl) anthranilate isomerase [AOC04\_10045] and anthranilate phosphoribosyltransferase [AOC04\_22465] within the genome of this strain.

Our genome analysis revealed presence of various PGPR traits in strain L10.10 including nitrogen fixation, hydrogen cyanide production, and phytohormone IAA biosynthesis, which highlight its potential as a promising PGPR. These beneficial traits also indicate the potential application of strain L10.10 in the development of eco-friendly biofertilizers which can assist in promoting soil fertility and crop yield.

#### **Nucleotide sequence accession number**

The complete chromosome sequence has been deposited in GenBank under the accession number CP012676

#### **Acknowledgements**

This work was supported by the University of Malaya High Impact Research Grants (UM-MOHE HIR Grant UM.C/625/1/HIR/MOHE/CHAN/14/1, Grant No. H-50001-A000027; UM-MOHE HIR Grant UM.C/625/1/HIR/MOHE/CHAN/01, Grant No. A-000001-50001) awarded to Kok-Gan Chan. The British Antarctic Survey (BAS) provided logistic support for the collection of the original soil sample from Lagoon Island. Peter Convey is supported by NERC core funding to the BAS 'Biodiversity, Evolution and Adaptation' Team, and this paper also

contributes to the Scientific Committee on Antarctic Research international programme 'Antarctic Thresholds – Ecosystem Resilience and Adaptation'.

## References

Ait Barka, E., Nowak, J., Clement, C. 2006. Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PsJN. Appl. Environ. Microbiol. 72, 7246–7252

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes, S., Glass, E.M., Kubal, M., Meyer, F., Olsen, G.J., Olson, R., Osterman, A.L., Overbeek, R.A., McNeil, L.K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G.D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A., Zagnitko, O., 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9, 75.

Brettin, T., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Olsen, G.J., Olson, R., Overbeek, R., Parrello, B., Pusch, G.D., Shukla, M., Thomason, J.A., Stevens, R., Vonstein, V., Wattam, A.R.,

Ee, R., Yong, D., Lim, Y.L., Yin, W.F. and Chan, K.G., 2015. Complete genome sequence of oxalate-degrading bacterium *Pandoraea vervacti* DSM 23571<sup>T</sup>. J. Biotechnol. 204, 5-6.

Goh, K.M., Chan, K.G., Yaakop, A.S. and Ee, R., 2015. Complete genome of *Jeotgalibacillus malaysiensis* D5<sup>T</sup> consisting of a chromosome and a circular megaplasmid. J. Biotechnol. 204, 13-14.

Lim, Y.L., Yong, D., Ee, R., Tee, K.K., Yin, W.F. and Chan, K.G., 2015. Complete genome sequence of *Serratia multitudinisentens* RB-25<sup>T</sup>, a novel chitinolytic bacterium. J. Biotechnol., 207, 32-33.

Nabti, E., Sahnoune, M., Ghoul, M., Fischer, D., Hofmann, A., Rothballer, M. *et al.* 2010. Restoration of growth of durum wheat (*Triticum durum* var. waha) under Saline conditions due to inoculation with the Rhizosphere Bacterium *Azospirillum brasilense*; NH and extracts of the marine alga; *Ulva lactuca*. J. Plant Growth Regul. 29, 6–22.

Overbeek, R., Olson, R., Pusch, G.D., Olsen, G.J., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Parrello, B., Shukla, M., Vonstein, V., Wattam, A.R., Xia, F., Stevens, R., 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. 42, D206–D214

Santi, C., Bogusz, D., Franche, C. 2013. Biological nitrogen fixation in non-legume plants. Ann. Bot., 111,743–767

Schippers, B., Bakker, A., van Peer, R. 1990. Beneficial and deleterious effects of HCN-producing pseudomonads on rhizosphere interactions. Plant Soil. 129,75–83

Xia, F., 2015. RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci. Rep. 5, 8365.

Zhao, Y. 2010. Auxin biosynthesis and its role in plant development. *Annu. Rev. Plant Biol.* 61, 49-64.

**Table 1:** Genome features of *Pseudomonas* sp. strain L10.10.

Attributes	Value
Genome size (bp)	5,172,488
Pseudogene	120
G+C content (%)	58.2
Total number of genes	4,639
Protein coding genes	4,425
Contigs	1
rRNA genes	25
tRNA genes	68