Journal of Biotechnology 253 (2017) 10–13

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Short communication

Genomic and phenotypic analyses of *Pseudomonas psychrotolerans* PRS08-11306 reveal a turnerbactin biosynthesis gene cluster that contributes to nitrogen fixation

Ruifang Liu[a,b,1], Yu Zhang[a,1], Ping Chen[a,1], Haiyan Lin[a,b], Guoyou Ye[a], Zhongwei Wang[a], Changwei Ge[a], Bo Zhu[c,d,*], Deyong Ren[a,**]

[a] State Key Lab for Rice Biology, China National Rice Research Institute, Hangzhou 310006, PR China
[b] Agricultural Genomes Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518120, China
[c] Zhejiang Province Key Laboratory of Plant Secondary Metabolism and Regulation, College of Life Science, Zhejiang Sci-Tech University, Hangzhou, China
[d] State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital of Medical College, Zhejiang University, China

ARTICLE INFO

Keywords:
Pseudomonas psychrotolerans
Turnerbactin
PacBio
Siderophore
Nitrogen fixation

ABSTRACT

Plant-microbe interactions can provide agronomic benefits, such as enhancing nutrient uptake and providing fixed nitrogen. The *Pseudomonas psychrotolerans* strain PRS08-11306 was isolated from rice seeds and can enhance plant growth. Here, we analyzed the *P. psychrotolerans* genome, which is ~5 Mb, with 4389 coding sequences, 77 tRNAs, and 7 rRNAs. Genome analysis identified a cluster of turnerbactin biosynthetic genes, which are responsible for the production of a catecholate siderophore and contribute to nitrogen fixation for the host. Analysis of the transcription factor mutant *ΔrpoS*, which does not express this gene cluster, confirmed the relationship between the gene cluster and siderophore production. The nitrogen fixation characteristics of the cluster were confirmed in a plant growth-promoting experiment. The annotated full genome sequence of this strain sheds light on the role of *P. psychrotolerans* PRS08-11306 as a plant beneficial bacterium.

1. Background

Symbiotic bacteria must survive inside host cells (Compant et al., 2010); however, bacteria require iron and in plant cells, proteins such as ferritin bind free iron atoms (Chu et al., 2010). To combat iron limitation, some bacteria produce siderophores, low-molecular weight compounds that have high affinity for Fe^{3+}, to take up iron from minerals (Chu et al., 2010). Previous work on shipworm endosymbionts identified turnerbactin, a tricatecholate siderophore, in the nitrogen-fixing bacteria *Teredinibacter turnerae* (Han et al., 2013). In this study, we report that the turnerbactin of *P. psychrotolerans* may be involved in nitrogen fixation.

The diverse *P. psychrotolerans* species (Spiers et al., 2000) occurs widely in nature and was first isolated from small animals in 2004 (Hauser et al., 2004). The first whole-genome sequence from a *P. psychrotolerans* strain isolated from copper coins was released in 2012 (Santo et al., 2012). Other studies have sequenced *P. psychrotolerans* strains from a clinical sample (Simmon et al., 2006), and from diseased rice (Adorada et al., 2013). In 2003, Xie et al. isolated several *Pseudomonas* spp., including *P. psychrotolerans* PRS08-11306, from rice seeds that were collected in the Philippines (Xie et al., 2003).

Emerging research has revealed the importance of plant-microbe interactions for crop production and spurred the identification of beneficial bacteria. For example, plants infected with *P. psychrotolerans* PRS08-11306 showed enhanced growth (Xie et al., 2003). Therefore, we selected this strain for whole-genome sequencing and genome sequence analysis.

2. DNA extraction and whole-genome sequencing

We amplified the 16S rRNA sequence from PRS08-11306 and confirmed that this strain should be classified as *P. psychrotolerans* (Fig. 1). *P. psychrotolerans* PRS08-11306 was inoculated overnight in Nutrient Broth (BD, USA) at 30 °C with shaking at 180 rpm. Genomic DNA was isolated from 2.5 ml of culture, using the Takara MiniBEST Bacteria Genomic DNA Extraction Kit (Takara, Dalian, China). The

* Corresponding author at: Zhejiang Province Key Laboratory of Plant Secondary Metabolism and Regulation, College of Life Science, Zhejiang Sci-Tech University, Hangzhou, China.
** Corresponding author.
E-mail addresses: bzhu@zstu.edu.cn (B. Zhu), rendeyong@caas.cn (D. Ren).
[1] Authors contributed equally.

http://dx.doi.org/10.1016/j.jbiotec.2017.05.012
Received 20 February 2017; Received in revised form 14 May 2017; Accepted 16 May 2017
Available online 18 May 2017
0168-1656/

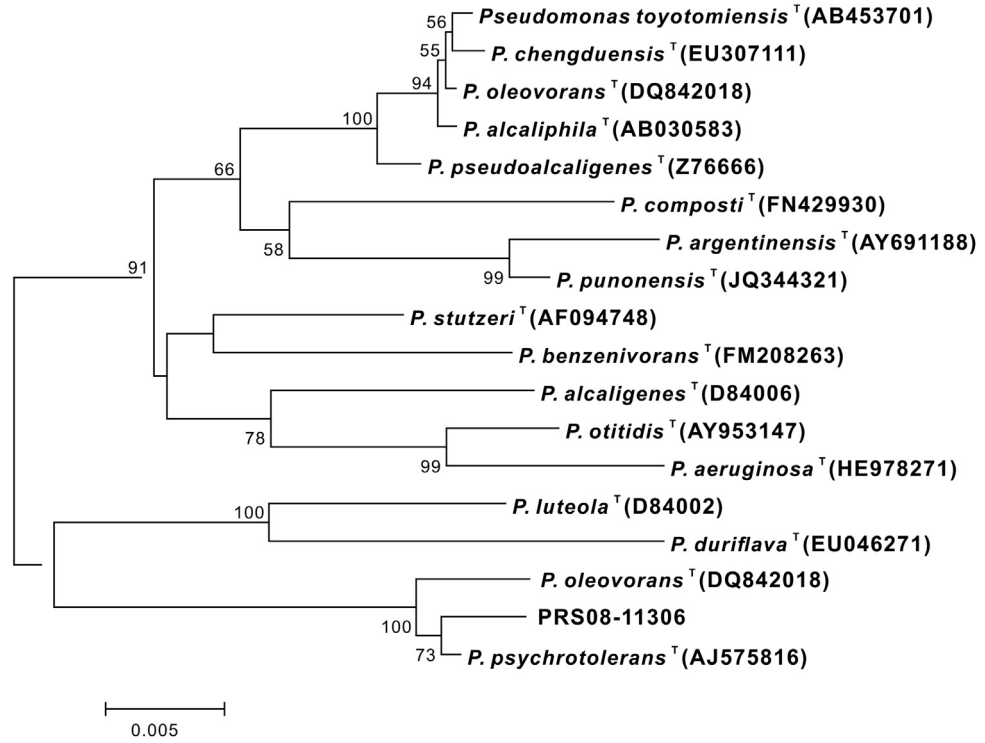

Fig. 1. Phylogenetic relationships based on 16S rRNA gene sequences were determined by the Maximum Likelihood method. Bootstrap confidence values were obtained using 100 resamplings. The tree shows the position of PRS08-11306 and selected type species of the genus *Pseudomonas*. Numbers at nodes indicate percentages of occurrence in 100 bootstrapped trees; only values greater than 50% are shown. Bar, 0.005 substitutions per site.

Table 1
Genomic features of *P. psychrotolerans* PRS08-11306.

Feature	Value
Genome size	5,386,170 bp
DNA coding	4,716,357 bp
DNA G+C	64.79%
Chromosome	1
Plasmid	1
Total genes	4925
Protein coding genes	4820
RNA genes	105
Genes with functional prediction	3989
Genes assigned to COGs	3544
Genes with Pfam domains	4120
Genes with signal peptides	446
Genes with transmembrane helices	1086
Genes related to secondary metabolism	1124
Genes related to antibiotic resistance	313

quality of the extracted DNA was tested with a Qubit 2.0 fluorometer (Life Technologies, MA, USA) and a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, MA, USA). The PacBio platform (Pacific Biosciences, Menlo Park, CA, USA) was used to perform whole-genome sequencing, which produced around 550 Mb of sequence, with 85-fold average coverage.

3. Genome assembly and annotation

After quality control, the PRS08-11306 genome was *de novo* assembled by using the Hierarchical Genome Assembly Process (HGAP), which can be accessed through the SMRT Analysis Portal, version 2.1.1. PBJelly v14.1.14 was used to fill and reduce as many captured gaps as possible, to produce a draft genome (English et al., 2012). CheckM with default parameters was used to test the completeness of the assembled genome (Parks et al., 2015). The genome annotation was done with Rapid Annotations using Subsystems Technology (Overbeek et al., 2014). Additional analysis was carried out using NCBI's UniProt database (http://www.ncbi.nlm.nih.gov/), Clusters of Orthologous Groups (Tatusov et al., 2003), Kyoto Encyclopedia of Genes and Genomes (Kanehisa and Goto, 2000), and Gene Ontology terms (Ashburner et al., 2000). Antibiotic and secondary metabolite analysis was done by searching against the antiSMASH database (Weber et al., 2015).

4. Genome statistics and general features

The genome of *P. psychrotolerans* PRS08-11306 is 5,386,170 bp with a G+C content of 64.8%. The completeness and contamination of this genome sequence was 98.53% and 0.90% after running CheckM, indicating the high quality of the assembled genome. RAST annotation predicted 4925 CDSs and of these, 3989 encode proteins with predicted functions (Fig. S1) and 3544 could be assigned to a COG category. The most abundant COG category was "Signal transduction mechanisms"

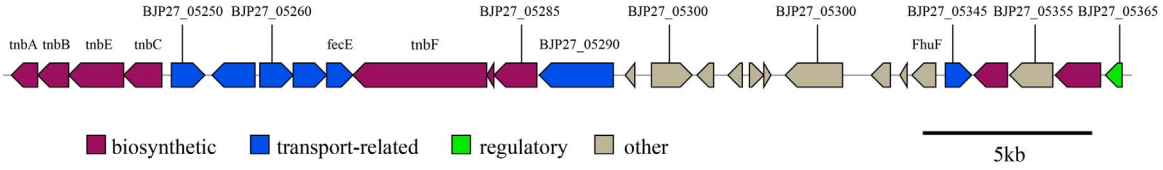

Fig. 2. Overview of the turnerbactin biosynthetic gene cluster in PRS08-11306. Organization of the genes involved in turnerbactin biosynthesis.