Genome Resource of *Ancylobacter pratisalsi* E130^T: A Novel Plant-Growth-Promoting Bacterium Isolated from the Rhizosphere

e-Xtra*

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Abstract

Ancylobacter pratisalsi sp. nov. strain E130^T is a Gram-negative, nonmotile, aerobic, and rod-shaped bacterium that was recently isolated from the rhizosphere of *Plantago winteri* Wirtg. from a natural salt meadow. This strain was described as novel species in genus *Ancylobacter*; however, information about its complete genome has yet not been reported. In this study, its genome was completely sequenced by PacBio SMRT cell platform, analyzed, and compared with other selected complete genome sequences of *Ancylobacter* to elucidate its potential plant growth promotion abilities. The genomic analysis revealed that the genome of strain E130^T consists of one circular DNA chromosome of 4,618,530 bp with a GC content of 66% and one plasmid of 159,741 bp with a GC content of 64.13%. The entire genome contains 4,322 predicted coding genes, 49 transfer RNAs, and 6 ribosomal RNA genes. Genome analysis identified a siderophore natural product biosynthesis cluster, which produces fusca-chelin. Knockout of several key genes in this cluster significantly reduces the plant-growth-promotion ability of the strain E130^T. In addition to plant-growth promotion, the strain E130^T can grow well on 5% NaCl (wt/vol), indicating that this strain is a potential bioresource for successful production of economic crops in alkaline soil.

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Keywords

bacterial pathogens, bioinformatics, biotechnology, microbe-genome sequencing, plant stress and abiotic disorders, rhizobial interactions

Genome Announcement

Ancylobacter is a newly named genus, which was originally proposed as genus *Microcycylus* (Raj 1983). Since 2005, this genus has been classified to the family *Xanthobacteraceae*. Members of the genus *Ancylobacter* are Gram-negative, rod-shaped, aerobic bacteria, including eight different species (Suarez et al. 2017), widely exhibiting its application prospect in different environmental conditions such as soil bioremediation of dichloromethane (Firsova et al. 2009), detoxification and degradation of halogenated aliphatic compounds in water sediments (van den Wijngaard et al. 1992), and as a plant-growth-promoting root endophyte in rice (Banik et al. 2016; van den Wijngaard et al. 1992). These findings suggest that *Ancylobacter* spp. may be suitable candidates as contaminated soil bioremediation and plant-growth-promoting agents.

Recently, Suarez et al. (2017) isolated isolate E130 from the rhizospheric soil of *Plantago winteri* Wirtg. Systematic analysis suggested that this strain should be classified as a new species

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Table 1. Genome statistics of *Ancylobacter pratisalsi* E130^T

Attribute	Chromosome	Plasmid
Genome size (bp)	4,618,530	159,741
DNA G+C (%)	66.00	64.13
Total genes	4,301	170
Protein coding genes	4,163	159
RNA genes	59	0
Ribosomal RNAs	6	0
Transfer RNAs	49	0
Noncoding RNAs	4	0
Pseudogenes	79	11
Genes with genome islands	54	0
NCBI accession	CP048630	CP048631

named *Ancylobacter pratisalsi*. Multiple in vitro plant-growth-promoting abilities of strain E130^T were tested, including 1-aminocyclopropane-1-carboxylate as sole nitrogen source, indole-3-acetic acid and siderophore biosynthesis, phytate mobilization, phosphate solubilization, and the detection of a marker gene for nitrogen fixation (Suarez et al. 2017). These results strongly suggest that this strain may have some potential application values on economically important plants. Therefore, whole-genome sequencing of strain E130^T was carried for molecular characterization and in vivo application to test its plant growth promotion ability.

Extraction of genomic DNA from strain E130^T was carried out as described previously (Moré et al. 1994). Whole-genome sequencing was performed by using PacBio sequencing (Pacific Biosciences, Menlo Park, CA, U.S.A.). Sequence runs for four single-molecule real-time (SMRT) cells were performed on the PacBio RS II sequencer with a 120-min movie time/SMRT cell. In total, 483,731 reads were obtained after sequencing, with an N₅₀ value of 6,339 bp and average read length of 4,668.5 bp, consisting of 2,258,311,723 bases in total. SMRT Analysis portal version 2.1 was used for read filtering and adapter trimming, with default parameters, and postfiltered data of 700 Mb (approximately 120× coverage) on each cell or strain with an average read length of 6.5 kb were considered for further assembly. The genome was then de novo assembled using HGAP assembly protocol, which is available with the SMRT Analysis packages and accessed through the SMRT Analysis Portal version 2.1. PBJelly V14.1.14 was used to fill and reduce as many captured gaps as possible to produce upgraded draft genomes (English et al. 2012). The protein coding genes, transfer RNA, and ribosomal RNA annotations were done by NCBI Prokaryotic Genome Annotation Pipeline (Pruitt et al. 2007). Clusters of orthologous groups of proteins and Kyoto Encyclopedia of Genes and Genomes were used to classify the predicted genes and pathways (Kanehisa and Goto 2000; Tatusov et al. 2003). Genes with signal peptides and transmembrane helices were identified using the TMHMM server and SignalP server, respectively (Krogh et al. 2001; Petersen et al. 2011). Genomic islands (GIs) were detected by applying IslandViewer, which mostly used GI detection algorithms IslandPick, SIGI-HMM, and IslandPath-DIMOB (Langille and Brinkman 2009). The secondary metabolite clusters, antibiotic resistance clusters, and toxins were predicted by comparing with antiSMASH and CARD, respectively (Jia et al. 2016; Weber et al. 2015). Genome comparison among TMS7^T and *Ancylobacter* genomes was conducted using average nucleotide identity (ANI), which was calculated by an ANI calculator (Varghese et al. 2015).

Genome assembly of strain E130^T revealed one circular chromosome with a size of 4,618,530 bp and one plasmid of 159,741 bp. In total, 4,163 coding sequences were predicted. Additional features of this genome are shown in Table 1. To access the similarity among strain E130^T and other closely related organisms, all available sequenced *Ancylobacter* genomes from all of the species were downloaded from NCBI and compared against strain E130^T. The genome similarity with E130^T ranged from 84.7 to 89.6% (Supplementary Table S1). The huge difference suggested that *A. pratisalsi* may have some unique features in its genome.

Interestingly, genome analysis using antiSMASH (Weber et al. 2015) predicted the existence of one gene cluster involved in fuscachelin biosynthesis (Supplementary Table S2). Fuscachelin is a kind of siderophore, which was first reported from the moderate thermophile *Thermobifida fusca* (Dimise et al. 2008). Although the molecular function of this siderophore is still unknown, many researchers have reported the strong link between bacterial siderophore and plant growth promotion (Pahari et al. 2017). To test the hypothesis that this cluster encodes genes related to plant

growth promotion, we examined the $\Delta G3A50_14880$ strain, which carries a deletion of a predicted core gene in this fuscachelin biosynthesis gene cluster, with a double homologous recombination method (Nie et al. 2020). The wild-type (WT) (E130^T) and the mutated ($\Delta G3A50_14880$) strains were inoculated on rice seedlings to confirm the role of the fuscachelin biosynthesis gene cluster in rice growth promotion. These tests were performed based on a previous report (Li et al. 2011), with five biological replicates. The rice plants were harvested after 30 days and growth parameters such as plant height, shoot fresh and dry weight, root fresh and dry weight, root length, and lateral root numbers were measured. The shoot weight (fresh and dry), root length, and lateral root numbers from WT and $\Delta G3A50_14880$ inoculated rice plants were significantly different from the control groups (Supplementary Fig. S1). The result confirmed that the fuscachelin biosynthesis gene cluster in the genome of *A. pratensis* E130^T plays an important role for plant growth promotion of rice; thus, it qualifies the strain E130^T as a plant-growth-promoting bacterium.

The complete genome presented here is the first available genome sequence of *A. pratensis*. The genome sequence of strain E130^T will provide new insight into the possible molecular mechanisms of this poorly studied plant beneficial bacterium.

Data Availability

This Whole-Genome Shotgun project has been deposited at GenBank under the accession numbers CP048630 and CP048631, BioProject PRJNA605092, and Sequence Read Archive SRR15244009.

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