



Genome Sequence of *Pseudomonas koreensis* CRS05-R5, an Antagonistic Bacterium Isolated from Rice Paddy Field

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INTRODUCTION

Pseudomonas koreensis, a new nominated Gram-negative bacterium was first reported and isolated from Korean agricultural soil (Kwon et al., 2003). CRS05-R5 (first reported as *Pseudomonas* sp.), which showed biocontrol ability against *Sitophilus oryzae* and *Acidovorax avenae* subsp. *avenae* (Liu et al., 2014), was first isolated from the rice rhizosphere in Heilongjiang province and reported in 2003 (Xie et al., 2003). Except for that, this species has been reported to produce the biosurfactant, which has biocontrol ability against *Phytophthora infestans* and *Pythium ultimum* (Hultberg et al., 2010a,b). These interesting features raise our attention on CRS05-R5. Recently, we sequenced the 16S rRNA sequence from CRS05-R5 and built the phylogenetic tree (Figure S1). Based on that, we confirmed that CRS05-R5 should be classified as *P. koreensis*. However, only one genome was sequenced (D26) and no detailed analysis was performed on this species. In this case, we did whole-genome sequencing on CRS05-R5, and tried to reveal the possible mechanism behind its antagonistic ability.

METHODS

Genomic DNA Isolation

Single colony of CRS05-R5 was inoculated into 5 ml NB (Nutrient Broth, BD, USA) at 30°C with 180 rpm vigorous shaking. 2.5 ml of culture broth was used to isolate the genomic DNA. DNA was extracted by Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). The quality of purified genomic DNA was tested by using NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, MA, USA) and Qubit 2.0 fluorometer (Life Technologies, MA, USA), respectively.

Whole Genome Sequencing and Annotation

Whole genome sequencing of CRS05-R5 was carried out by using PacBio RS II platform. Six hundred Megabytes raw data was obtained with 100X coverage. After quality control, genome assembly was *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. Genome annotation was later done by using RAST annotation system (Overbeek et al., 2014). The completeness of the assembled genome was tested by CheckM with default parameters (Parks et al., 2015). In addition, GO and COG programs were used to do further functional analysis of all annotated ORFs (Ashburner et al., 2000; Tatusov et al., 2000). Specifically, since antagonistic bacteria, especially

fluorescent pseudomonads, usually compete with other microorganisms by using secondary metabolism (Haas and Défago, 2005), genes, which are predicted to be involved in secondary metabolism were compared with DoBISCUIT database (Ichikawa et al., 2013). The circular genome map of CRS05-R5 including all predicted ORFs with COG functional assignments, rRNA, tRNA, G+C content, and GC skew information were generated using Circos (Krzywinski et al., 2009), as shown in **Figure S2**.

Genome Comparison

Genome comparison among CRS05-R5 and other fully sequenced *Pseudomonas* genomes were carried out by using ANI (average nucleotide identity) and AF (alignment fraction), which were calculated by ANIcalculator (Varghese et al., 2015), and Circos (Krzywinski et al., 2009). In order to find out the unique genes in CRS05-R5, all the fully sequenced *Pseudomonas* genomes were downloaded from NCBI (58 genomes). The protein sequences from CRS05-R5 were compared with the protein sequences from these 58 genomes with 40% identity as cutoff. The protein sequences which cannot find any homologs in these 58 genomes were classified as unique genes.

Direct Link to Deposited Data and Information to Users

This strain has been deposited in CGMCC with deposit number 1.15630. This genome sequencing project has been deposited at DDBJ/EMBL/GenBank under the Accession Number CP015852. The BioProject designation for this project is PRJNA322127.

INTERPRETATION OF DATA SET

General Genome Sequence Property

The total size of the genome is 5,991,224 bp and has a G+C content of 60.6%. The completeness and contamination of this genome is 99.86% and 0.05% after running the CheckM. These results indicate the high quality of assembled genome. A total of 5352 CDSs were predicted. Of these, 3832 could be assigned to a COG number. The most abundant COG category was “General function prediction only” (581 proteins) followed by “Signal transduction mechanisms” (547 proteins), “Amino acid transport and metabolism” (539 proteins), “Transcription” (448 proteins), and “Function unknown” (302 proteins). In addition, 82 RNAs including rRNA and tRNA were identified. All the genomic information was shown in **Table 1**.

Secondary Metabolism in CRS05-R5

Biosurfactants are amphiphilic compounds produced by microorganisms (Mulligan, 2005). This compound, which is produced by *Pseudomonas* spp., has been widely used in biocontrol (Debode et al., 2007). In CRS05-R5 genome, more than 800 genes are predicted to be involved in secondary metabolism by comparing with DoBISCUIT database (Ichikawa et al., 2013). Interestingly, we found one gene cluster, which is annotated as cyclic lipopeptides (CLPs), exists in CRS05-R5 (*arfABC*). CLP is a kind of biosurfactant, and has been proved to be important in antagonistic *Pseudomonas* sp. (Raaijmakers

TABLE 1 | Genome statistics.

| Features | Value |
|----------------------------------|-----------|
| Genome size(bp) | 5,991,224 |
| Contig numbers | 1 |
| G+C % | 60.6 |
| Protein-coding genes | 5352 |
| Protein with known function | 4363 |
| tRNA number | 76 |
| rRNA number | 6 |
| ncRNA number | 75 |
| Genes with signal peptides | 573 |
| Genes with transmembrane helices | 1187 |

et al., 2006). This information indicates that CRS05-R5 can be used as biocontrol agent.

Genome Comparison

Strikingly, we found even the ANI value between CRS05-R5 and *P. koreensis* D26 is only 92.5% (**Figure S3**). Also, the highest AF value is only 74.2% (**Table S1**). Indeed, 631 CDSs were predicted to be unique genes existing in CRS05-R5 genome. These results indicate the huge genome diversity among *Pseudomonas* strains, which is consistent with previous findings (Loper et al., 2012). Circos result found many unique genes in CRS05-R5 (**Figure 1**). To better understand the features of these genes, *Pseudomonas* database was used to deeply annotate these genes (Winsor et al., 2011). Except for a lot of hypothetical proteins, we found one gene cluster encoding fimbrial associated proteins only existing in CRS05-R5 (A8L59_09240–A8L59_09310). These genes have been reported to be critical for the initial stage of biofilm development (Wei and Ma, 2013). Except for this gene cluster, we also found three *rhs* genes exist as the unique genes in CRS05-R5 (A8L59_00750, A8L59_09890, and A8L59_11585). These genes have been found to be linked to the second type VI secretion cluster in *P. aeruginosa* (Jones et al., 2014). Also, these genes have been reported to mediate intercellular competition (Koskiniemi et al., 2013). More strikingly, one *rhs* gene is located in a unique gene cluster (A8L59_11555–A8L59_11600). Although, most of the genes are hypothetical proteins, we may infer that this cluster maybe related with secretions. Indeed, by searching with TMHMM Server v. 2.0, which is a transmembrane helices prediction database (<http://www.cbs.dtu.dk/services/TMHMM/>), we found that 3 genes (A8L59_11560, A8L59_11565, and A8L59_11600) have predicted transmembrane helices. Since biofilm and intercellular competition are very important for bacterial survival and adaptation, we infer that these genes may confer some fitness advantages for CRS05-R5 on the root of *Oryza sativa*. Also, we found one unique gene cluster (A8L59_18500–A8L59_18575), which encodes *wbpL* and other glycosyl transferase. These genes have been confirmed to be important in lipopolysaccharide formation (Rocchetta et al., 1998). These compounds have been proved to be important in plant roots colonization (Duijff et al., 1997) as well as induction of systemic resistance against some

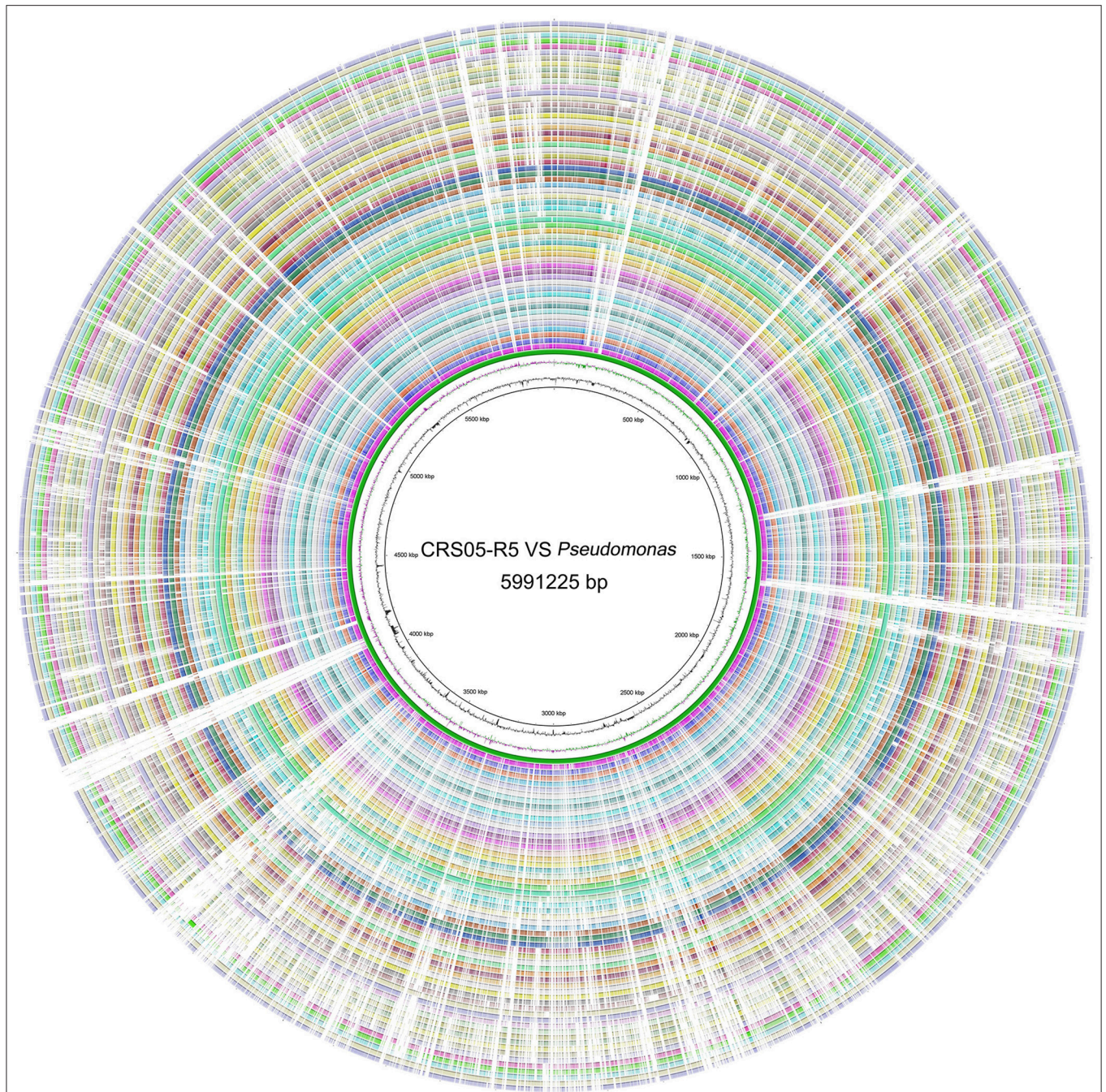


FIGURE 1 | Circular map of the chromosome of *P. koreensis* CRS05-R5 and other fully sequenced *Pseudomonas* genomes. The tracks from the inside to outside: GC Content, GC Skew, *P. koreensis* CRS05-R5, *P. koreensis* D26, *P. aeruginosa* B136-33, *P. aeruginosa* c7447 m, *P. aeruginosa* DK2, *P. aeruginosa* LES431, *P. aeruginosa* LESB58, *P. aeruginosa* M18, *P. aeruginosa* MTB-1, *P. aeruginosa* NCGM2.S1, *P. aeruginosa* PA1, *P. aeruginosa* PA1R, *P. aeruginosa* PA7, *P. aeruginosa* PAO1-VE13, *P. aeruginosa* PAO1-VE2, *P. aeruginosa* PAO1, *P. aeruginosa* PAO581, *P. aeruginosa* RP73, *P. aeruginosa* SCV20265, *P. aeruginosa* UCBPP-PA14, *P. denitrificans* ATCC 13867, *P. entomophila* L48, *P. fluorescens* A506, *P. fluorescens* Pf0-1, *P. fluorescens* SBW25, *P. fulva* 12-X, *P. mendocina* NK-01, *P. mendocina* ymp, *P. monteilii* SB3078, *P. monteilii* SB3101, *P. poae* RE1-1-14, *P. protegens* CHAO, *P. protegens* Pf-5, *P. putida* DOT-T1E, *P. putida* F1, *P. putida* GB-1, *P. putida* H8234, *P. putida* HB3267, *P. putida* KT2440, *P. putida* NBRC 14164, *P. putida* ND6, *P. putida* S16, *P. putida* W619, *P. resinovorans* NBRC 106553, *Pseudomonas* sp. TKP, *Pseudomonas* sp. UW4, *Pseudomonas* sp. VLB120, *P. stutzeri* A1501, *P. stutzeri* ATCC 17588, *P. stutzeri* CCUG 29243, *P. stutzeri* DSM 10701, *P. stutzeri* DSM 4166, *P. stutzeri* RCH2, *P. syringae* pv. *phaseolicola* 1448A, *P. syringae* pv. *syringae* B728a, *P. syringae* pv. *tomato* DC3000, *P. brassicacearum* subsp. *Brassicacearum* NFM421, *P. fluorescens* F113.

plant pathogens (Leeman et al., 1995). All these results strongly indicate the biocontrol potential of CRS05-R5 strain.

AUTHOR CONTRIBUTIONS

HL performed the experiments and write the manuscript. SH and RL helped in data analysis. PC, CG, and BZ coordinated the work and drafted the manuscript. LG conceived the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fmicb.2016.01756/full#supplementary-material>

Table S1 | The fraction of orthologous genes (Alignment Fraction, AF) value between CRS05-R5 and other Pseudomonas genomes.

Figure S1 | Phylogenetic relationships based on 16S rRNA gene sequences were determined by the neighbor-joining method with the program package MEGA 6.0 (Tamura et al., 2013). Bootstrap confidence values were obtained using 1000 resamplings. The tree shows the positions of strains CRS05-R5 and other selected Pseudomonas strains. Numbers at nodes indicate percentages of occurrence in 1000 bootstrapped trees; only values >50% are shown. Bar, 0.005 substitutions per site.

Figure S2 | Graphical map of the chromosome genome of P. koreensis CRS05-R5. From the outside to the center: genes on forward stand and Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, ncRNAs black), GC content, GC skew.

Figure S3 | Heatmap of Average Nucleotide Identity (ANI) between CRS05-R5 and all the other sequenced Pseudomonas genomes.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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