Complete genome sequence of *Paenibacillus yonginensis* DCY84<sup>T</sup>, a novel plant Symbiont that promotes growth via induced systemic resistance

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**Abstract**

This article reports the full genome sequence of *Paenibacillus yonginensis* DCY84<sup>T</sup> (KCTC33428, JCM19885), which is a Gram-positive rod-shaped bacterium isolated from humus soil of Yongin Forest in Gyeonggi Province, South Korea. The genome sequence of strain DCY84<sup>T</sup> provides greater understanding of the *Paenibacillus* species for practical use. This bacterium displays plant growth promotion via induced systemic resistance of abiotic stresses.

**Keywords:** *Paenibacillus yonginensis* DCY84<sup>T</sup>, Genome, PacBio, Plant growth promoting rhizobacteria (PGPR)

**Introduction**

Various *Paenibacillus* species constitute a large group of facultative anaerobic endospore-forming Gram-positive bacteria that are extensively distributed in nature. Ash et al. proposed that members of ‘group 3’ within the genus *Bacillus* should be transferred to the genus *Paenibacillus*, for which they proposed *Paenibacillus polymyxa* as the type species [1]. Since that time, 174 different type species have been described.

Members of the genus *Paenibacillus* are well known as PGPR, together with *Azotobacter, Azospirillum, Pseudomonas, Acetobacter*, and *Burkholderia* [2]. While many new species from the genus *Paenibacillus* have been reported [3], the type species *Paenibacillus polymyxa* [4] is considered a PGPR that is widely used in sustainable agriculture and environmental remediation because of its multiple functions [2, 5]. Coupled with many plant species, some *Paenibacillus* species have been developed as biofertilizers or biocontrol agents and have been used effectively in the control of plant-pathogenic fungi, bacteria, and nematodes [5–7]. *P. yonginensis* DCY84<sup>T</sup> was isolated from a decomposed humus mixture in South Korea and its plant growth promotion traits have been characterized in vitro [8]. This strain is capable of inducing the defense response of *Arabidopsis* against several abiotic stresses [9]. Genome sequencing of *P. yonginensis* DCY84<sup>T</sup> was conducted to obtain additional insights into the physiological characteristics involved in microbe-plant interactions and to facilitate better understanding of the molecular basis of these traits.

**Organism information**

**Classification and features**

*Paenibacillus yonginensis* DCY84<sup>T</sup> was isolated from a decomposed humus mixture collected from Yongin province. It is a Gram-positive bacterium that can grow on Tryptic soy broth agar at 28 °C. Cells of strain DCY84<sup>T</sup> are rod-shaped with a diameter ranging from 0.7–0.9 μm and length ranging from 3.4 to 4.7 μm. Growth occurs under aerobic conditions with an optimum growth temperature at 25–30 °C and a temperature range of 15–40 °C, general features of strain DCY84<sup>T</sup> were presented in Table 1. Phylogenetic tree highlighting the position of *Paenibacillus yonginensis* DCY84<sup>T</sup> and phylogenetic inferences were obtained using the maximum-likelihood method (Fig. 1). Cell
morphology was examined using scanning electron microscopy (Fig. 2).

**Genome sequencing information**

*Genome project history*

*P. yonginensis* DCY84<sup>T</sup> was selected for genome sequencing because we observed the presence of a unique compatible solute for plant protection from biotic stress and potential plant growth promoting activity with rice in reclaimed paddy soil and *Panax ginseng* C.A.Mey, respectively. The complete genome sequence has been deposited in the NCBI sequencing read archive under NCBI BioProject PRJNA306396 with BioSample SAMN04419545 and overall sequencing project information was presented in Table 2. Sequencing, annotation, and analysis were performed at LabGenomics (Seongnam, Republic of Korea).

**Growth conditions and genomic DNA preparation**

For growth and genomic DNA preparation, *P. yonginensis* DCY84<sup>T</sup> (KCTC 33428<sup>T</sup>=JCM 19885<sup>T</sup>) was grown in DSMZ medium 1 (Nutrient Agar) at 28 °C. DNA was isolated from 0.5–1 g of cell paste using the JetFlex genomic protocol as recommended by the manufacturer. For genome sequencing and assembly, the draft genome of *P. yonginensis* DCY84<sup>T</sup> was generated using the PacBio platform following the manufacturer’s instructions.

### Table 1 Classification and general features of *Paenibacillus yonginensis* DCY84<sup>T</sup>

| MIGS ID | Property | Term | Evidence Code |
|---------|----------|------|---------------|
|         | Classification | Domain Bacteria | TAS [17] |
|         | Phylum | Firmicutes | TAS [18, 19] |
|         | Class | Bacilli | TAS [20] |
|         | Order | Bacillales | TAS [21, 22] |
|         | Family | Paenibacillaceae | TAS [21, 23] |
|         | Genus | Paenibacillus | TAS [15] |
|         | Species | *Paenibacillus yonginensis* | TAS [8, 9] |
|         | Strain | DCY84<sup>T</sup> | TAS [8, 9] |
|         | Gram stain | positive | IDA |
|         | Cell shape | rod | IDA |
|         | Motility | motile | IDA |
|         | Sporulation | spore production | IDA |
|         | Temperature range | 15–40 °C | IDA |
|         | Optimum temperature | 30 °C | IDA |
|         | pH range; Optimum | 5–9; 8 | IDA |
|         | Carbon source | D-Xylose, D-ribose, D-glucose and others | TAS [8] |
|         | Habitat | humus soil | IDA |
|         | Salinity | 0.5–4.5% NaCl | IDA |
|         | Oxygen requirement | Aerobic | IDA |
|         | Carbon source | glucose, lactose | TAS [8] |
|         | Biotic relationship | Free-living | IDA |
|         | Pathogenicity | Non-pathogenic | NAS |
|         | Source material identifiers | KCTC 33428<sup>T</sup>, JCM 19885<sup>T</sup> | TAS [8] |
|         | Geographic location | South Korea: Gyeonggi province | IDA |
|         | Sample collection | September 2013 | IDA |
|         | Latitude | 37.314 N | IDA |
|         | Longitude | 127.268 W | IDA |
|         | Altitude | 131.37 m | IDA |

Evidence codes: IDA inferred from direct assay, TAS traceable author statement (i.e., a direct report exists in the literature), and NAS non-traceable author statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [24].
Genome sequencing and assembly
Sequencing produced 74,264 reads with an average length of 7828 bp, which was assembled using the de novo HGAP implemented within the analysis pipeline SMRT Analysis 2.2 (Pacific Biosciences, CA, USA). Ambiguous base and inserted/deleted regions between the PacBio assembled and preassembled high quality draft sequences were manually corrected using consensus sequences for final assembly. Long reads were selected as the seed sequences for constructing preassemblies, and the other short reads were mapped to the seeds using BLASTR software for alignment, which corrected errors in the long reads and thus increased the accuracy rating of bases. The sequencing run yielded 581,398,217 filtered and sub-read bases and a total of 113,985,693 preassembled bases were used for deep sequencing. tRNA and rRNA genes were identified by tRNAscan-SE version 1.3 [10] and RNAmmer version 1.2 [11]. The ORFs were predicted using Glimmer 3.02 and the annotation of predicted genes was conducted using Blastall 2.2.26. Protein coding genes were annotated based on the COGs database.

Genome annotation
The purpose of the present study was to develop a better understanding of the P. yonginensis DCY84ᵀ genetic background to develop more effective utilization of the strain. COGs analysis of strain DCY84ᵀ is shown in Fig. 3 and the number of genes associated with the 22 general COGs functional categories presented in Table 3. The analysis of the full P. yonginensis DCY84ᵀ genome in
The *iaaM* gene, also a gene responsible for IAA synthesis, siderophore production, phosphate transporter, phosphonate cluster, antimicrobial production, and synthesis of the volatile organic compound *bdhA* are present in the *P. yonginensis* DCY84\(^T\) genome. These genes corroborate with our physiological results demonstrating plant growth promotion and induced systemic resistance in the plant symbiont [9, 10].

**Insights from the genome sequence**

The completed *P. yonginensis* DCY84\(^T\) genome consists of a single circular chromosome of 4,985,901 bp, with a GC content of 51.01\%, which is similar to most *Paenibacillus* strains (45–54\%) as reported previously [12] (Fig. 4). The genome size of the strain DCY84\(^T\) (4.985 Mb) is smaller than the other sequenced members of genus *Paenibacillus* including *P. polymyxa* CF05 (5.76 Mb), and *P. mucilaginosus* 3016 (8.74 Mb) [13]. Full genome of DCY84\(^T\) was annotated by following NCBI prokaryotic genome annotation pipeline [14]. A total of 4498 genes were predicted for the genome, including 4233 coding sequences (94.1\% of total genes) and 147 pseudo genes. Nucleotide content and gene count levels of the chromosome were summarized in Table 4. More detail annotation of the strain DCY84\(^T\) was available in Additional file 2: Table S5. Most of selected *Paenibacillus* strain was reported to have plant growth promoting factor traits. The summary features of DCY84\(^T\) and referred strains are shown on Additional file 1: Table S1 below, including the genome accession number, genome size, GC content, annotation information, protein, Gene, Pseudo gene. The COGs analysis of strain DCY84\(^T\) and other closely related *Paenibacillus* strains was provided on Additional file 1: Table S2 (direct plant growth promoting factors) and Additional file 1: Table S3 (indirect plant growth promoting factors). The genome of *P. yonginensis* DCY84\(^T\) and *P. polymyxa* M1 were visualized in Additional file 3: Figure S1 by the comparison using the Artemis software and ACT [15]. Strain DCY84\(^T\) increased nutrient availability by producing several hydrolyzing enzymes, amino acid transporter proteins (Additional file 1: Table S4). Moreover, Strain DCY84\(^T\) treatment can induce plant defense mechanism mediated by ABA signal under salinity stress.

**Extended insights**

Genome analysis showed that *P. yonginensis* DCY84\(^T\) contained many genes related to the stress response, such as IAA, choline, glutamate decarboxylase and malate transporters, potassium uptake protein, heat shock proteins, chaperone proteins, and sugar transporters.
These genes most likely allow the strain to cope with different environmental stresses. Experimentation and additional analysis of these genes may help to elucidate the mechanisms mediating the stress response and facilitate the development of *P. yonginensis* DCY84\(^T\) as a biofertilizer. When the strain DCY84\(^T\) was used as a treatment for early sprouting rice seeds, several genes responsible for primary metabolism were upregulated in the rice root, which could be related to PGPR. These results indicate that *P. yonginensis* DCY84\(^T\) might have the potential for application in industrial biotechnology as a producer of miscellaneous hydrolases.

This is the first report describing the genome sequence of *P. yonginensis* DCY84\(^T\). When coated on sprouting rice seeds or seedlings directly on paddy soil, strain DCY84\(^T\) and silica zeolite complex were shown to enhance rice yield and also increase GABA content in brown rice. Treatment was also shown to induce systemic stress resistance responses in rice and *Arabidopsis* under heavy metal and salty conditions. Furthermore, the sequence of *P. yonginensis* DCY84\(^T\) provides useful information and may contribute to agricultural applications of *Paenibacillus* genera in practical biotechnology. Rice yield was affected by the amount of strain DCY84\(^T\) administered during the early sprouting stage. Silica zeolite complex and strain DCY84\(^T\) treatment inhibited the occurrence of fungal infection, and also enhanced rice quality. Silica zeolite complex and two treatments with strain DCY84\(^T\) resulted in the highest head rice levels (86.8%) compared to a one-time treatment of DCY84\(^T\) (67.9%), and without strain DCY84\(^T\) treatment (46.4%). The PGPR treatment enhanced head rice levels by 40.4% [16]. Strain treatment also enhanced nitrogen uptake and increased levels of stored nitrogen in the rice grain, indicating that the strain DCY84\(^T\) enhanced plant nitrogen utilization with less nitrogen fertilizer application. The most important parameters for economic rice value are head rice rate and good appearance; strain DCY84\(^T\) treatment enhanced both the rice quality and reduced commercial nitrogen fertilizer usage.

| Code | Value | Percentage | Description |
|------|-------|------------|-------------|
| J    | 170   | 4.02       | Translation, ribosomal structure and biogenesis |
| A    | 0     | 0.00       | RNA processing and modification |
| K    | 360   | 8.50       | Transcription |
| L    | 233   | 5.50       | Replication, recombination and repair |
| B    | 0     | 0.00       | Chromatin structure and dynamics |
| D    | 30    | 0.71       | Cell cycle control, cell division, chromosome partitioning |
| V    | 73    | 1.72       | Defense mechanisms |
| T    | 217   | 5.13       | Signal transduction mechanisms |
| M    | 190   | 4.49       | Cell wall/membrane/envelope biogenesis |
| N    | 61    | 1.44       | Cell motility |
| U    | 21    | 0.50       | Intracellular trafficking, secretion, and vesicular transport |
| O    | 109   | 2.58       | Posttranslational modification, protein turnover, chaperones |
| C    | 121   | 2.86       | Energy production and conversion |
| G    | 431   | 10.18      | Carbohydrate transport and metabolism |
| E    | 260   | 6.14       | Amino acid transport and metabolism |
| F    | 93    | 2.20       | Nucleotide transport and metabolism |
| H    | 99    | 2.34       | Coenzyme transport and metabolism |
| I    | 104   | 2.46       | Lipid transport and metabolism |
| P    | 163   | 3.85       | Inorganic ion transport and metabolism |
| Q    | 31    | 0.73       | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 334   | 7.89       | General function prediction only |
| S    | 278   | 6.57       | Function unknown |
| –    | 1372  | 32.41      | Not in COGs |
| Total| 4750  | 112.21     | |

\(^{a}\)The percentage is based on the total number of protein coding genes in the annotated genome

\(^{b}\)The total does not correspond to 4498 CDS because some genes are associated with more than one COG functional categories
Conclusion

The DCY84\textsuperscript{T} strain was isolated from a decomposed humus mixture. Phylogenetic analysis based on the 16S rRNA gene confirmed its affiliation to the genus *Paenibacillus*. G + C content, COGs, and average nucleotide identities are presented. The genomic features of strain DCY84\textsuperscript{T} are consistent with the plant growth promoting activity of this strain, including IAA production, phosphate solubilizing activity, and siderophores production. In addition, DCY84\textsuperscript{T} induced systemic stress resistance mechanisms in rice and *Arabidopsis* under heavy metal and salty conditions.

Additional files

- **Additional file 1:** Table S1. Genome comparison of strain DCY84\textsuperscript{T} and closest *Paenibacillus* strains. Table S2. COGs analysis of direct plant growth promoting traits. Table S3. COGs analysis of indirect plant growth promoting traits. Table S4. Some important genes annotated on strain DCY84\textsuperscript{T} genome. (DOCX 81 kb)
Additional file 2: Table S5. Annotation of the Paenibacillus yonginensis DCY84\(^{1}\) genome. (XLSX 443 kb)

Additional file 3: Figure S1. Comparative genome analysis of \emph{P. yonginensis} DCY84\(^{1}\) and \emph{P. polymyxa} M1 using the Artemis software and ACT. (TIFF 16717 kb)

**Abbreviations**
bddA: 2,3-butanediol synthase; COGs: Clusters of Orthologous Groups of proteins; HGAP: Hierarchical Genome Assembly Process; IAA: Indole-3-acetic acid; isdA: Tryptophan monooxygenase; ORFs: Open Reading Frames; PGPR: Plant Growth Promoting Rhizobacteria; SMRT: Single Molecule, Real-Time

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**Authors’ contributions**
YJK designed the study, carried out the genome analysis, and drafted the manuscript. JS performed DNA isolation, electron microscopy, the phylogenetic analysis for taxonomic study and corrected the manuscript. JWS and CHK carried out the sequencing and helped to draft the manuscript. ESC and SS participated in the study design. DCY coordinated. All authors read and approved the final manuscript.

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

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