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Genome Sequence and Comparative Genome Analysis of *Pseudomonas syringae* pv. *syringae* Type Strain ATCC 19310

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Pseudomonas syringae pv. *syringae* (*Psy*) is a major bacterial pathogen of many economically important plant species. Despite the severity of its impact, the genome sequence of the type strain has not been reported. Here, we present the draft genome sequence of *Psy* ATCC 19310. Comparative genomic analysis revealed that *Psy* ATCC 19310 is closely related to *Psy* B728a. However, only a few type III effectors, which are key virulence factors, are shared by the two strains, indicating the possibility of host-pathogen specificity and genome dynamics, even under the pathovar level.

Keywords: *Pseudomonas syringae* pv. *syringae* ATCC 19310, type strain, comparative genomics, type III effector

The genus *Pseudomonas* is a large group belonging to the gamma subclass of proteobacteria that can utilize a variety of organic compounds as energy sources and produce secondary metabolites [9, 12, 17]. *Pseudomonas syringae* is a globally distributed phytopathogenic, host-specific, and hemibiotrophic bacterial pathogen of a variety of plant species, commonly causing disease symptom such as leaf spots and necrosis, fruit specks and scabs, flower wilting, twig die-back, and branch and trunk cankers [1]. Using a range of molecular, biochemical, and physiological approaches, 57 pathovars have been identified [6]. *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000) was the first pathovar to have its genome that was completely sequenced [5], followed by those of the snap bean pathogen *P. syringae* pv. *syringae* (*Psy*) B728a [10] and the kidney bean pathogen *P. syringae* pv. *phaseolicola* 1448A (*Pph* 1448A) [13]. *Psy* B728a can exist as an epiphyte on the surface of bean leaves before invading the apoplast [15]. *Psy* is a typical bacterial pathogen causing leaf spots, canker, and blossom blights in many economically important plant species [11]. Although *Psy* has been comprehensively studied, the full genome sequence of the type strain had not been determined. In this study, we report the first draft sequence of the *Psy* type strain ATCC 19310 (=KCTC12500) and present a comparative genomic analysis with the sequenced strains *Pto* DC3000, *Psy* B728a, and *Pph* 1448A.

Psy ATCC 19310 was grown in King's B broth at 30ºC. Cells were harvested and resuspended in 50 mM EDTA (pH 8.0) before lysing with achromopeptidase (5 mg/ml) and lysozyme (10 mg/ml). Genomic DNA was isolated according to the manufacturer's instructions (Promega, WI, USA) and used for library construction and genomic sequencing with an Illumina HiSeq 2000 system at the Human Derived Material Center of KRIBB, Daejeon, South Korea.

To test the hypersensitive response (HR; rapid localized cell death at the site of infection), 4-week-old tobacco (*Nicotiana benthamiana*) seedlings, a non-host plant, were infiltrated with bacterial suspensions of *Psy* ATCC 19310 (OD_{600} = 0.01) in the leaf tissue.

Typical disease symptoms of *Psy* were observed in challenged leaves of lima bean (Fig. 1A, left panel). *Psy*

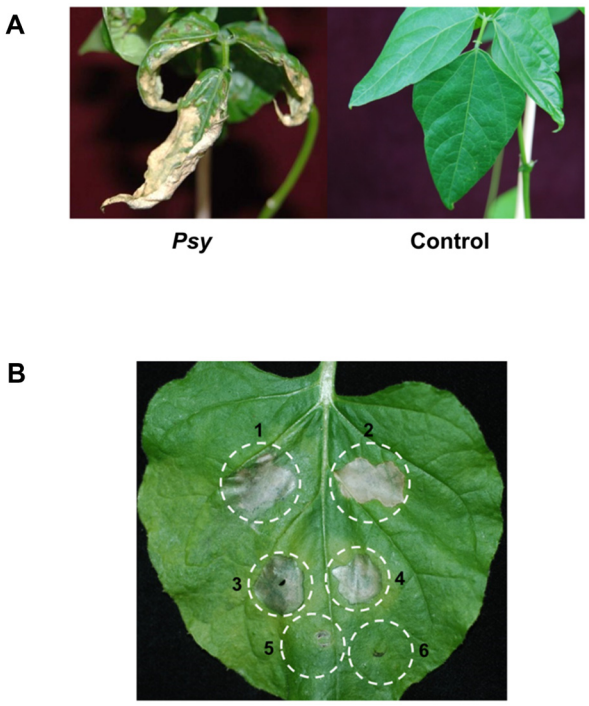

Fig. 1. Disease symptoms of *Psy* in lima bean and the HR in *Nicotiana benthamiana*.

(**A**) Three-week-old seedlings of lima bean were sprayed with suspensions of *Psy* (10^7 CFU/ml) or control. The representative pictures were taken 10 days after the pathogen was applied. (**B**) Suspensions of *Psy* ATCC 19310 (1), *Psy* B728a (2), *Pph* 1448A (3), *Pto* DC3000 (4), and *Pta* 6605 (5), as well as the buffer control (6) were infiltrated into leaves of *Nicotiana benthamiana*. The number around the circle indicates infiltrated strains and control as described above. Hypersensitive cell death was observed in areas inoculated with *Psy* ATCC 19310, *Psy* B728a, *Pph* 1448A, or *Pto* DC3000.

ATCC 19310 was infiltrated into tobacco leaves and the HR appeared in the treated area within 72 h (Fig. 1B). Three other pathogens, namely, *Psy* B728a, *Pph* 1448A, and *Pto* DC3000, elicited an HR similar to *Psy* ATCC 19310, whereas no response was detected after infiltration of *P. syringae* pv. *tabaci* 6605 (*Pta* 6605) or the buffer control within 72 h (Fig. 1B).

The *Psy* ATCC 19310 genome was sequenced using a HiSeq 2000 system (Illumina, CA, USA) and an Ion PGM system (Life Technologies, CA, USA). A total of 34,390,868 paired-end reads (101 nt; 3.47 Gb total) were produced by the Illumina system using a fragment library of ca. 330 bp insert size. Preprocessing and *de novo* assembly were performed using the CLC Genomics Workbench ver. 5.5 (CLC Bio), which represented 51 scaffolds (98 contigs over 200 bp) out of 2.75 Gb of quality-trimmed and filtered reads. The total scaffold length, maximum scaffold length, and N_{50} were 6,080,222 bp, 846,064 bp, and 382,372 bp, respectively.

A 3 kb library was constructed using the SOLiD mate-pair library kit to improve the scaffold structure. Conversion of the SFF file into 474,768 paired-end reads (37.9 Mb, average length 79.8 bp) was performed using the CLC Genomics Workbench ver. 6.5. SSPACE Premium ver. 2.3 (BaseClear) was used to produce higher-level scaffolds from the CLC assembly and Ion PGM mate-pair reads. The final assembly resulted in 18 scaffolds with maximum scaffold length and N_{50} of 2,753,553 bp and 1,563,801 bp, respectively. The total scaffold length including N's was 6,101,756 bp with a G+C content of 58.7%. The assembled sequences were automatically annotated using the RAST server [4]. There were 5,267 protein-coding genes, and approximately half (51.6%) were classified into subsystems. Scaffold sequences were inspected for length, sequencing coverage, the presence of genes encoding Rep proteins, and homology to known plasmid sequences of *P. syringae* pathovars. No putative plasmid sequences were identified from the assembly (data not shown).

The genome sequence of *Psy* ATCC 19310 was deposited at DDBJ/EMBL/NCBI under the accession number AYTM00000000. The version described in this study was the first, AYTM01000000. Raw sequence files were also uploaded at SRA under the accession number SRS504702.

We compared the draft genome sequence of *Psy* ATCC 19310 with three previously sequenced *P. syringae* strains, namely, *Pto* DC3000, *Psy* B728a, and *Pph* 1448A. The draft sequences of seven further strains were downloaded from NCBI as representatives of each pathovar and used for comparative analysis. MUMmer-based whole-genome alignment (http://mummer.sourceforge.net/) revealed that the sequence of *Psy* ATCC 19310 aligned at a relatively high level with *Psy* B728a, showing minimal genomic rearrangements when compared with *Pto* DC3000 or *Pph* 1448A (Fig. 2A).

BLAST-based nucleotide level similarities between *Psy* ATCC 19310 and ten other *P. syringae* strains (including three complete sequences and seven representative draft sequences) were visualized using the BRIG [2]. Prior to the analysis, 18 scaffold sequences of *Psy* ATCC 19310 were joined into one pseudomolecule that had been aligned along with the complete genome sequence of *Psy* B728a using MUMmer. The result showed regions associated with a low G+C content that was *Psy*-specific or *Psy* ATCC 19310-specific (Fig. 2B).

DNA relatedness was previously used as an indicator to

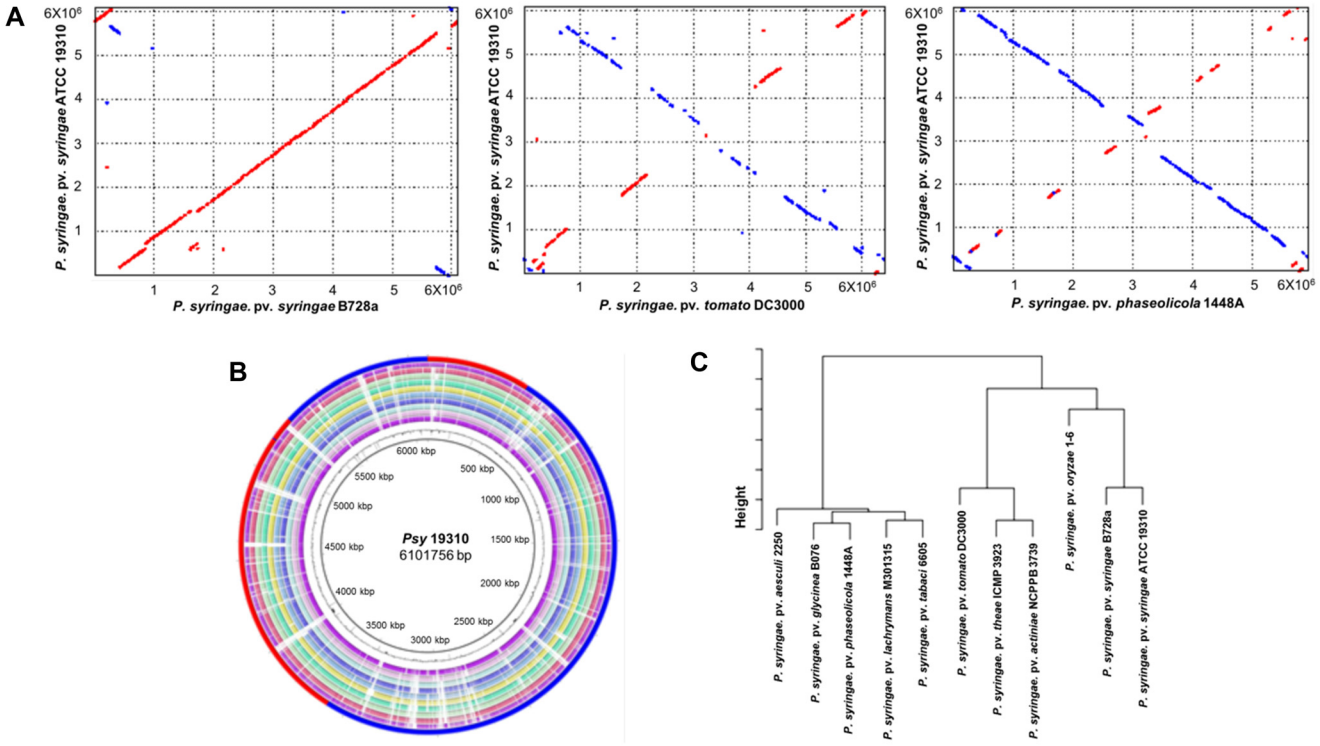

Fig. 2. Comparative genome analyses of *Psy* ATCC 19310 with other *Pseudomonas syringae* strains.
(**A**) Whole-genome alignment of *Psy* ATCC 19310 genome sequences (Y-axis) against three complete sequences of *P. syringae* strains (X-axis). From left to right: *Psy* B728a (CP000075), *Pto* DC3000 (AE016853), and *Pph* 1448A (CP000058). (**B**) Circular representation of BLAST similarities using concatenated scaffolds of *Psy* ATCC 19310 as a query against ten genomes of *Pseudomonas syrinage* strains. Circles represent (inner to outer): %G+C, *Psy* B728a, *Pto* DC3000, *Pph* 1448A, *P. syringae* pv. *aesculi* 2250 (*Pas* 2250), *P. syringae* pv. *lachrymans* M301315 (*Pla* M301315), *P. syringae* pv. *oryzae* 1-6 (*Por* 1-6), *P. syringae* pv. *glycineas* B076 (*Pgl* B076), *P. syringae* pv. *actinidiae* NCCPB 3739 (*Pac* NCCPB 3739), *P. syringae* pv. *tabaci* 6605 (*Pta* 6605), and *P. syringae* pv. *theae* ICMP3923 (*Pth* ICMP3923). (**C**) Hierarchical clustering of 11 strains using ANI values.

identify a new prokaryote species [8]; however, in this era of genomics, the average nucleotide identity (ANI) between a given pair of genomes has become the preferred option. ANI for 11 *P. syringae* genome sequences was calculated using the BLAST algorithm and MUMmer alignment using the software tool JSpecies [16]. The ANI value between *Psy* ATCC 19310 and *Psy* B728a was greater than 95% (Table 1), indicating that the two strains are the same species. Hierarchical clustering based on ANI values identified at least four independent groups among 11 strains of *P. syringae*, with *Psy* ATCC 19310 and *Psy* B728a classified into the same group (Fig. 2C).

The type III secretion system (T3SS) of *P. syringae* and many other proteobacteria injects effector proteins into the cells of plants and animals [7]. The *hrp* genes that encode T3SS and effector proteins of *P. syringae* are important in eliciting the HR in non-host plants [14]. The HR was present on tobacco leaves (Fig. 1B), indicating that *Psy* ATCC 19310 elicits the non-host resistance and is a pathogenic strain on the certain host. Potential type III effectors that are key virulence factors from *Psy* ATCC 19310 were investigated using a BLAST search and a database for *P. syringae* (http://pseudomonas-syringae.org/pst_func_gen2.htm). Twelve effectors for *Psy* ATCC 19310 were present, which was a smaller number in comparison with *Pph* 1448A (17), *Psy* B728a (27), and *Pto* DC3000 (41). All four strains showed very different distributions of effectors, and only AvrE1 was present in each (Fig. 3). The ANI values and hierarchical clustering indicated that *Psy* ATCC 19310 and *Psy* B728a were closely related (Fig. 2C and Table 1); however, the pattern of effectors in their genomes was not similar (Fig. 3). Among the effector genes, *hopA2*, *hopW1*, *hopAZ1*, and *hopBC1* were only present in *Psy* ATCC 19310 (Fig. 3). Although *Psy* ATCC 19310, *Psy* B728a, and *Pph* 1448A are pathogenic to bean, the complement of effector genes is different in each strain. In addition to our result, the diversity of type III effector repertoires was easily reported in *P. syringae* [3]. The

Table 1. Average nucleotide identity (ANI) values among 11 *Pseudomonas syringae* strains.

Pathovar name	1[b]	2	3	4	5	6	7	8	9	10	11
1. *P. syringae*. pv. *theae* ICMP3923	100	**95.7**	88.4	88.4	**98.9**	88.6	87.7	88.4	88.5	87.9	87.7
2. *P. syringae*. pv. *tomato* DC3000[a]	**95.7**	100	88.3	88.2	**95.7**	88.6	88.1	88.3	88.5	88	87.7
3. *P. syringae*. pv. *lachrymans* M301315	88.4	88.3	100	**98.9**	88.5	**98.2**	87.3	**98.2**	**97.9**	89.7	89.6
4. *P. syringae*. pv. *tabaci* 6605	88.4	88.1	**98.9**	100	88.4	**98.2**	87.3	**98.2**	**98**	89.7	89.5
5. *P. syringae*. pv. *actinidiae* NCPPB 3739	**98.9**	**95.7**	88.5	88.4	100	88.6	87.7	88.6	88.5	87.9	87.7
6. *P. syringae*. pv. *glycinea* B076	88.5	88.5	**98.2**	**98.2**	88.6	100	87.4	**99.3**	**97.8**	89.7	89.4
7. *P. syringae*. pv. *oryzae* 1-6	87.7	88.1	87.3	87.3	87.8	87.5	100	87.2	87.3	87.6	87.4
8. *P. syringae*. pv. *phaseolicola* 1448A[a]	88.4	88.3	**98.2**	**98.2**	88.5	**99.2**	87.2	100	**97.8**	89.7	89.4
9. *P. syringae*. pv. *aesculi* 2250	88.5	88.5	**97.9**	**97.9**	88.5	**97.8**	87.3	**97.8**	100	89.8	89.6
10. *P. syringae*. pv. *syringae* B728a[a]	87.9	88	89.7	89.7	87.9	89.8	87.5	89.7	89.8	100	**95.1**
11. *P. syringae*. pv. *syrinage* ATCC 19310[a]	87.7	87.7	89.6	89.5	87.7	89.5	87.3	89.5	89.6	**95.1**	100

[a]Complete sequences.

[b]The numbers indicate the pathovar.

ANI values >95%, a de *facto* cutoff value for the identical species, are highlighted in bold.

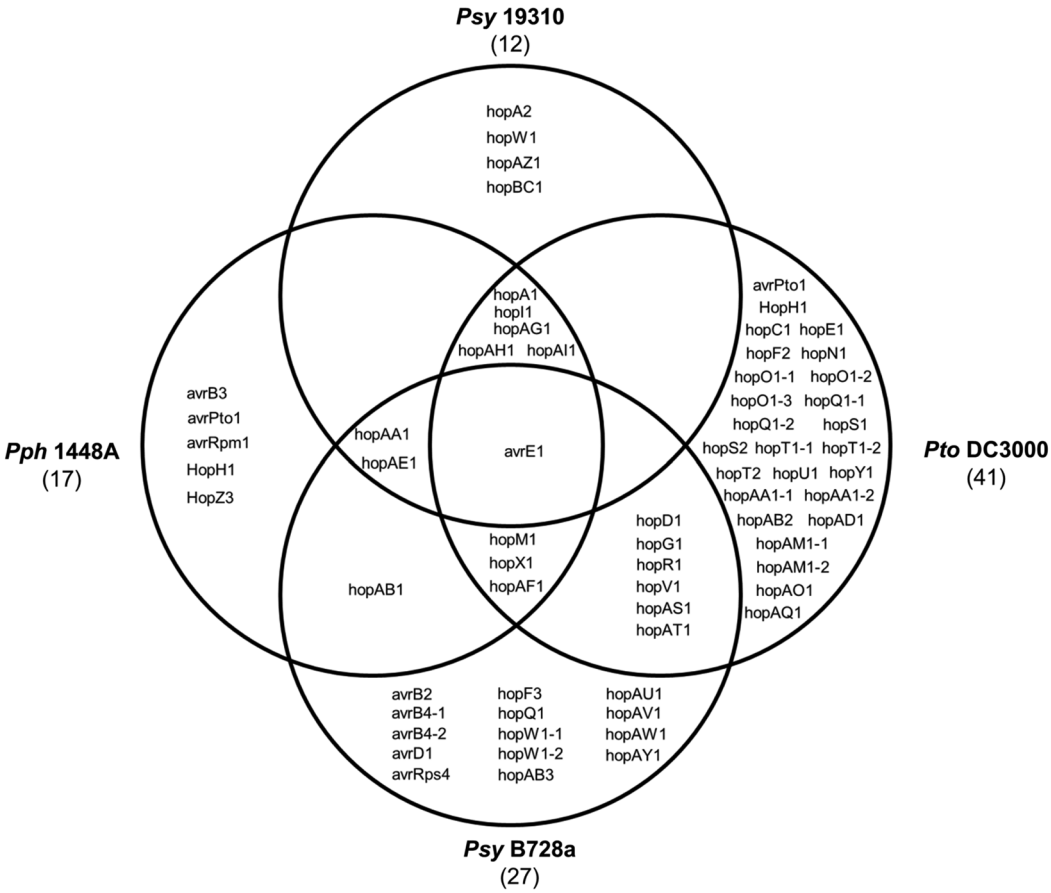

Fig. 3. Venn diagram of the type III effector gene components of *Psy* ATCC 19310 (top), *Psy* B728a (bottom), *Pto* DC3000 (right), and *Pph* 1448A (left).

Genes that are conserved among all four strains are shown in the middle of the diagram. The number below the strain name indicates the total number of type III effectors.

findings of our study suggest that there are many different combinations of effectors conferring *P. syringae* virulence in the same plant. In order to elucidate the correlation between certain effectors and their pathogenicity, further study will be definitely needed at this moment.

In conclusion, we report the draft genome sequence of the *Psy* type strain ATCC 19310. It is closely related to *Psy* B728a, except for the complement of type III effectors, which are also different to those found in *Pph* 1448A and *Pto* DC3000. Our results broaden the current knowledge of *P. syringae* virulence factors and their candidates for further genetic and functional characterization.

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