The Complete Genome Sequence of the Nicotine-Degrading Bacterium Shinella sp. HZN7

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BACKGROUND

Nicotine is a natural alkaloid that is very toxic to humans. To eliminate the harmful effects of nicotine in the environment, biological methods employing microbes to degrade nicotine are required (Brandsch, 2006; Liu et al., 2015). Shinella sp. HZN7 can degrade nicotine efficiently via the variant of a pyridine and pyrrolidine pathways (VPP; Ma et al., 2013; Qiu et al., 2014, 2015). The main intermediates in this pathway include 6-hydroxy-nicotine, 6-hydroxy-N-methylmyosmine, 6-hydroxypseudooxynicotine, 6-hydroxy-3-succinoyl-pyridine, and 2,5-dihydroxypyridine. This strain is the first nicotine-degrading bacterium to be isolated from the genus Shinella.

The genus Shinella was established in 2006 within the “Rhizobiaceae group” of the Alphaproteobacteria (An et al., 2006). Six species were assigned to this genus namely S. daejeonensis, S. fusca, S. granuli, S. kummerowiae, S. yambaruensis, S. zoogloeoides. However, most strains in this genus have not been identified at the species level. Shinella spp. have been isolated from various environmental samples, such as active sludge, zooplankton gut, soils, and water. They also exhibit a range of functional diversity, such as nitrogen fixation (Lin et al., 2008), assimilation of phosphite (Poehlein et al., 2016), and degradation of the toxic pollutants, 4-aminobenzenesulfonate (Biala et al., 2014), chlorothalonil (Liang et al., 2011), and pyridine (Bai et al., 2009). Until now, only three Shinella draft genomes have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/genome/genomes/32494). To further understand the molecular mechanism of nicotine degradation and advance the potential biotechnological applications of Shinella strains, we present the first complete genome sequence of Shinella sp. HZN7 and its features.

MATERIALS AND METHODS

Bacterial Strain and DNA Purification

Shinella sp. HZN7 was isolated from the active sludge of a wastewater-treatment system of a pesticide manufacturer in Hangzhou City, China. This bacterium was cultured aerobically in LB medium at 30°C with 100 µg/mL ampicillin. Genomic DNA from Shinella sp. HZN7 was extracted and purified using a QIAamp DNA Mini Kit (Qiagen, Germany). The concentration of genomic Genomic DNA was measured using a Qubit 2.0 Fluorometer (Thermo Scientific, USA). Purity of DNAs samples (UV A260/A280) was assessed using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA).
TABLE 1 | Genomic features of Shinella sp. HZN7.

| Features                         | Chromosome | pShin-01 | pShin-02 | pShin-03 | pShin-04 | pShin-05 | pShin-06 | Total          |
|----------------------------------|------------|----------|----------|----------|----------|----------|----------|---------------|
| Genome size (bp)                 | 4,678,597  | 620,539  | 445,803  | 409,126  | 222,555  | 155,026  | 147,896  | 7,354,253     |
| G + C content (%)                | 65.2       | 66.2     | 65.5     | 65.3     | 65.1     | 58.5     | 62.1     |               |
| Total genes                      | 4480       | 571      | 389      | 388      | 200      | 144      | 149      |               |
| Protein coding genes             | 4334       | 556      | 376      | 379      | 187      | 137      | 144      |               |
| RNA genes                        | 64         | 0        | 0        | 0        | 0        | 0        | 0        |               |
| Mobile genetic elements          | 85         | 1        | 4        | 3        | 1        | 30       | 9        |               |
| GenBank accession No.            | CP015736   | CP015737 | CP015738 | CP015739 | CP015740 | CP015741 | CP015742 |               |

TABLE 2 | Summary of gene cluster involved in nicotine degradation in Shinella sp. HZN7.

| Gene locus tag | Size (amino acids) | Genes with predicted function | Function of most similar gene product(s) | Source accession no. and identity |
|---------------|--------------------|-------------------------------|------------------------------------------|---------------------------------|
| shn_30145     | 157                | Transposase                   | –                                        | –                               |
| shn_30195     | 391                | Para-nitrophenol 4-monooxygenase | 6-Hydroxy-3-succinoylpyridine 3-monooxygenase, VppD | AIH15770 (100%)                |
| shn_30205     | 671                | Hypothetical protein          | 6-Hydroxypseudoxynicotine oxidase, Pno   | WP_024899819 (100%)          |
| shn_30230     | 736                | Chemotaxis protein            | Methyl-accepting chemotaxis protein, MCP | Q00986 (47%)                  |
| shn_30235     | 344                | Putrescine/spermidine ABC     | –                                        | –                               |
|               |                    | Transporter substrate-binding protein | –                                        | –                               |
| shn_30250     | 358                | DDE endonuclease              | –                                        | –                               |
| shn_30255     | 226                | Tetr family transcriptional regulator | –                                        | –                               |
| shn_30260     | 344                | Putrescine/spermidine ABC     | –                                        | –                               |
|               |                    | Transporter substrate-binding protein | –                                        | –                               |
| shn_30265     | 266                | ABC transporter permease       | –                                        | –                               |
| shn_30270     | 301                | ABC transporter permease       | –                                        | –                               |
| shn_30275     | 357                | Spermidine/putrescine ABC     | –                                        | –                               |
|               |                    | Transporter ATP-binding protein | –                                        | –                               |
| shn_30280     | 210                | Carbarnoylsarcosine amidase   | Maleamate amidase, VppG                  | AIH15798 (100%)                |
| shn_30285     | 342                | Leucyl aminopeptidase         | 2,5-DHP dioxygenase, VppE                 | AIH15799 (99%)                 |
| shn_30290     | 260                | Alpha/beta hydrolase          | N-formylmaleic acid deformylase, VppF     | AIH15800 (100%)                |
| shn_30295     | 249                | Asp/Glu racemase              | Maleate isomerase, VppH                   | AIH15801 (100%)                |
| shn_30300     | 465                | Aldehyde dehydrogenase        | 4-Aminobutanal dehydrogenase             | Q6DBY7 (39%)                   |
| shn_30305     | 437                | Hypothetical protein          | 6-Hydroxy-nicotine oxidase, NctB          | AGS16700                       |
| shn_30310     | 551                | Transposase                   | –                                        | –                               |
| shn_30325     | 527                | Hypothetical protein          | Nicotine hydroxylase large subunit VppAL | AIH15806 (100%)                |
| shn_30330     | 155                | (2Fe-2S)-binding protein      | Nicotine hydroxylase small subunit VppAS | AIH15807 (100%)                |
| shn_30370     | 477                | Transposase                   | –                                        | –                               |

Genome Sequencing and Assembly

The genome of strain HZN7 was sequenced using the PacBio RSII platform. A 20-kb DNA library was constructed according to the manufacturer’s instructions and sequenced using single-molecule realtime (SMRT) sequencing technology with the P6 DNA polymerase and C4 chemistry. The sequences from two
SMRT cells were assembled with SMRT Pipe version 2.1.1 using the hierarchical genome-assembly process (HGAP). The reads were de novo assembled and polished using the PacBio software HGAP3/Quiver (Chin et al., 2013).

**Genome Annotation**

The coding sequences (CDSs) were predicted using the Prokaryotic Genome Annotation Pipeline (PGAP) version 3.2 software on NCBI (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The locus tag prefix was set as “shn.” Additional gene prediction and annotation was performed using the Rapid Annotation Subsystems Technology (RAST) server (Aziz et al., 2008). CRISPR finder (http://crispr.u-psud.fr/Server/) was used for identifying CRISPR/Cas systems (Grissa et al., 2007).

**RESULTS**

**Genome Features**

After quality control, ~4 Gb of data was obtained with a 550-fold average coverage. A total of 7,354,253 bp genome sequence was assembled. The features for the complete genome sequence of *Shinella* sp. HZN7 are summarized in Table 1. The complete genome is composed of one circular chromosome and 12 circular plasmids (designated as plasmid pShin-01 to pShin-12) with an average GC content of 64.8%. The whole genome contains 6954 genes, including 6694 coding sequences, 355 rRNAs, 3165 rRNAs, 2385 rRNAs, 51 tRNAs, 4 ncRNA, and 196 pseudo genes. Interestingly, 215 mobile genetic elements were predicted, including 96 transposases, 33 integrases, and 86 conjugative transfer proteins. Moreover, one CRISPR gene cluster was identified by the CRISPR finder tool. To the best of our knowledge, there are no bacterial strains containing up to 12 circular plasmids. Previous reports have shown that *Shinella zoogloeoides* strain BC026 contained 3 or more 200 kb megaplasmids (Bai et al., 2010) and *Shinella* sp. DD12 contained at least seven plasmids (Poehlein et al., 2016). These results indicate that the possession of multiple plasmids is a common feature in the genus *Shinella*.

**Nicotine-Degrading Gene Cluster**

Our previous study showed that the novel 6-hydroxy-nicotine oxidase, NctB, was responsible for the degradation of 6-hydroxy-nicotine to 6-hydroxy-pseudo-oxy-nicotinic (Qiu et al., 2014). The nctB gene (locus tag shn_30305) was found on the plasmid pShin-05. The nctB gene, as well as genes homologous to vppA (nicotine hydroxylase gene), vppE (2,5-dihydroxy-pyridine dioxygenase gene) from *Ochrobactrum* sp. strain SIY1 (Yu et al., 2015) and pno (6-hydroxy-pseudo-oxy-nicotinic oxidase gene) from *Agrobacterium tumefaciens* S33 (Li et al., 2016), appeared in an 50 kb region of DNA with a GC content of 56.6%. The predicted genes (shn_30145 to shn_30370) in this cluster and their characterized homolog are summarized in Table 2. This cluster was not found in three other *Shinella* draft genomes (http://www.ncbi.nlm.nih.gov/genome/genomes/32494). In addition, two transposase genes flanked this cluster of DNA, indicating that it may have been acquired by horizontal gene transfer.

In conclusion, we present the first complete genome of *Shinella* sp. HZN7. We hope this will facilitate a deeper understanding of the molecular mechanism of nicotine degradation via the VPP pathway, and provide a reference genome for genus *Shinella*.

**ETHICS STATEMENT**

This article does not contain any studies with human participants or animals performed by any of the authors.

**DATA ACCESS**

The complete genome sequence of *Shinella* sp. HZN7 has been deposited at the GenBank/EMBL/DDBJ under the accession numbers CP015736-CP015748. The strain is available from the China Center for Type Culture Collection under the accession no CCTCC M 2013060 or from Dr. JQ at Nanjing Agricultural University.

**AUTHOR CONTRIBUTIONS**

JQ and ZL conceived and designed the research; YY, JZ, and HW performed experiments and analyzed data; JQ, YM, JH, and ZL analyzed data; JQ and ZL wrote the manuscript; all authors commented on the manuscript and approved the contents.

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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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