

Draft genome sequence of silver nanoparticle producing bacterium *Paenibacillus anseongense* MAH-34^T

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은나노입자를 생성하는 *Paenibacillus anseongense* MAH-34^T의 유전체 서열 분석

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A bacterial strain MAH-34^T was isolated from rhizosphere soil of magnolia flower tree. The isolate can grow on R2A agar/broth at 10~37°C with optimum growth at 28~30°C and pH 7.0 without NaCl supplement. The MAH-34^T was responsible for the silver nanoparticle (AgNP) production via hydrolysis of AgNO₃. Thus, to know about the nitrate reductase genes, the genome analysis of *Paenibacillus anseongense* MAH-34^T was carried out. The assembled genome of MAH-34^T consists of 42 scaffolds with a total of 8,647,101 bp with N50 and N75 values of 553,491 and 230,930, respectively. The DAN G + C content was 46.0 mol%. Additionally, the genome annotation also shows the nitrate and/or nitrite reductase genes using the Rapid Annotation using Subsystem Technology (RAST), which may be responsible for the production of silver nanoparticle (AgNP) through the hydrolysis of AgNO₃.

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Previously, the genus *Paenibacillus* was considered as a common member of genus *Bacillus*. However, based on its unique phenotypic and genotypic characteristics, the *Paenibacillus* was proposed as a separate genus in 1993 (Ash *et al.*, 1993). Members of this genus are Gram-stain variable, aerobic, motile rods, and endospore-forming. Currently, the genus contained 296 species and 4 sub-species (<https://lpsn.dsmz.de>), which have been isolated from various environmental samples including soil, clinical specimens, fresh and saltwater, flowers, forage, and insect larvae (Huang *et al.*, 2014; Siddiqi *et al.*, 2015, 2017; Sáez-Nieto *et al.*, 2017; Huq, 2020). Many research studies describe the importance of the members of the genus *Paenibacillus*

which produce various metabolites, catalyze a wide variety of synthetic reactions in both cosmetics and biofuel production. Therefore, the species of the genus *Paenibacillus* have achieved the importance in agriculture, industrial and medical applications (Konishi and Maruhashi, 2003).

The microbial reduction of valuable metal ions such as silver, platinum, gold, and palladium into metallic nanoparticles has been reported previously (Hennebel *et al.*, 2009; Lin *et al.*, 2014). Among these nanoparticles, the silver nanoparticles (AgNPs) are used progressively in various fields, including, food, biosensors, medical, consumer, health care, and industrial purposes due to their unique chemical and physical properties (Majdalawieh *et al.*, 2014; Singh *et al.*, 2015).

Therefore, here we report several nitrate reductase genes from the genome analysis of MAH-34^T, which may be responsible for the production of silver nanoparticles (AgNPs) via the hydrolysis of AgNO₃. The strain MAH-34^T was isolated from rhizosphere soil of magnolia tree in Anseong city Republic of Korea (37° 00' 39"N 127° 22' 79"E). The isolate grows well on R2A agar medium at 10~37°C (optimum growth at 28~30°C) with a pH range 6.0~9.5 (optimum pH 7.0) (Huq, 2020). The genomic and phylogenetic analysis put the strain MAH-34^T within the genus *Paenibacillus*.

Presently, the complete or draft genome sequence analysis plays a key role in the description of novel bacterial species and target gene identification. As the strain MAH-34^T was positive for the production of silver nanoparticles (AgNPs) by the hydrolysis of AgNO₃ (Huq, 2020). Therefore, to identify the nitrate and/or nitrite reductase genes, the strain MAH-34^T was subjected to whole-genome sequencing analysis.

The genomic DNA of strain MAH-34^T was then extracted and purified as described by Marmur (1961), with some modifications. A genomic library was constructed using a TruSeq DNA PCR-free library preparation kit (Illumina) according to

the manufacturer's instructions and sequenced on the Illumina HiSeq-XTen platform to generate 302-bp paired-end reads. High-quality sequence fragments (2,962,264 paired-end reads, total of 785 Mb, and 62-fold coverage of the genome) were then assembled using SOAPdenovo v. 3.10.1. Genome annotation and analysis were performed using the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAP, <http://www.ncbi.nlm.nih.gov/books/NBK174280/>) and RAST (Overbeek *et al.*, 2014). The project information is available from the Genomes OnLine Database.

The assembled genome of *Paenibacillus anseongense* strain MAH-34^T contained 42 scaffolds with a total of 8,647,101 bp (G + C content, 46.0%), an N50 value of 553,491 bp, an average sequencing depth of 62, and maximum contig lengths of 1,491,448 bp. The genome contains 7,720 coding genes, 132 RNA genes, and 99 pseudo-genes.

To find the nitrate reductase genes, the genome of MAH-34^T was annotated by RAST (Rapid Annotation Using Subsystem Technology). The annotated analysis shows a total of 22 nitrate and/or nitrite reductase genes. Among these 22 genes, only 4 genes have the RAST IDs (GO IDs) with one published gene [Nitrous-oxide reductase (NosZ)] as shown in Table 1. Thus, it is predicted that some of the genes may be responsible for the production of silver nanoparticles (AgNPs) via the hydrolysis of AgNO₃.

Data Availability

The draft genome sequence of MAH-34^T has been deposited at DDBJ/EMBL/GenBank under the accession number WSEM00000000. This strain is available from the Korean Agricultural Culture Collection, South Korea with the accession number KACC 19974^T and China General Microbiological Culture Collection Center with the accession number CGMCC1.16610^T as well as from the host institution (Chung Ang University,

Table 1. The list of nitrate/nitrite reductase of MAH-34^T

S.No	Abbreviations	Functional role	GO IDs	Literature
1	NorQ	Nitric-oxide reductase activation protein NorQ	GO:0005524, GO:0016887	None
2	NorB	Nitric-oxide reductase subunit B (EC 1.7.99.7)	GO:0016966	None
3	NorC	Nitric-oxide reductase subunit C (EC 1.7.99.7)	GO:0016966	None
4	NosZ	Nitrous-oxide reductase (EC 1.7.99.6)	GO:0050304	8 Publications

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적 요

목련 나무의 근권 토양에서 박테리아 균주 MAH-34^T를 분리하였다. 균주 MAH-34^T는 10~37°C의 온도, R2A 고체 및 액체 배지에서 자라고 28~30°C, pH 7.0, NaCl이 없을 때 가장 잘 배양되었다. 균주 MAH-34^T는 질산은(AgNO_3)의 가수 분해를 통해 은나노 입자(AgNP)를 생산하는 능력을 갖고 있었다. 이에 질산염 환원 효소 유전자에 대해 알기 위해 *Paenibacillus anseongense* MAH-34^T의 유전체 서열 분석을 수행하였다. MAH-34^T의 조립된 유전체 서열은 총 8,647,101 bp, N50 및 N75 값이 각각 553,491 및 230,930 인 42개의 스캐폴드로 구성되며, DNA G+C 함량은 46.0 mol%이었다. 또한 유전체 서열 주석은 RAST (Rapid Annotation using Subsystem Technology)를 사용하여 분석하였으며 그 결과로 AgNO_3 의 가수 분해를 통해 은나노 입자(AgNPs)의 생산을 담당할 수 있는 질산염 및 아질산염 환원 효소 유전자가 존재함을 보여주었다.

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